Human IL-1 alpha Platinum ELISA

PRODUCT INFORMATION & MANUAL

Enzyme-linked Immunosorbent Assay for quantitative detection of human IL-1 alpha

Catalog Number  REF  BMS243/2CE, BMS243/2TENCE

Σ  96 TESTS

For in-vitro diagnostic use. Not for therapeutic procedures.
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1. Intended Use

The human IL-1 alpha ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-1 alpha. **The human IL-1 alpha ELISA is for in vitro diagnostic use. Not for use in therapeutic procedures.**

2. Summary

The interleukin-1 (IL-1) species represent an important family of biologically active mono nuclear cell-derived proteins which are involved in inflammatory reactions and in immune responses. Two distinct human IL-1 species, IL-1 alpha and IL-1 beta, have been identified. They share similarities such as the same molecular weight, similar biological effects and the same receptors on target cells. IL-1 proteins are produced by macrophages, monocytes and various other cell types such as adult T cell leukemias, fibroblasts, epithelial or endothelial cells, neutrophils and astrocytes. Their biological properties include pyrogenicity, bone resorption, presentation of antigen to T cells and stimulation of B and T lymphocyte proliferation.

IL-1 alpha is an extracellular peptide of 17kDa, its activity has been demonstrated in various biological fluids including serum, synovial fluid, gingival fluid, amniotic fluid, sputum, cerebrospinal fluid, urine, and bronchoalveolar lavage (BAL) fluid.

Elevated serum or blood levels of IL-1 alpha have been found in patients with total hip replacement/arthroplasties, in patients with recently diagnosed IDDM, in case of several carcinomas such as head and neck cancer, pancreatic cancer and thyroid cancer, in experimental acute pyelonephritis, in acute viral hepatitis and in septic shock. Both elevations in serum levels and joint fluids (synovial fluids) are detected in rheumatoid arthritis. IL-1 alpha elevation is a marker for dental diseases such as pulpal inflammation and infections of the root canals. Pulmonary disorders are accompanied by plasma and BAL elevations of IL-1 alpha, e.g. cystic fibrosis, systemic sclerosis. Increased plasma and CSF levels are found in patients with schizophrenia. Blood levels of newborn with systemic infection during the neonatal period are significantly higher than in controls. High concentration of IL-1 alpha in the cervical mucus of pregnant women are found to be involved in defense mechanism against ascending infections.

Significantly elevated concentrations in gingival crevicular fluid in subjects with periodontitis are detected. Urinary levels of IL-1 alpha correspond to disease and therapy response in bladder cancer.

For literature update refer to [www.Thermo Fisher.com](http://www.Thermo Fisher.com)

3. Principles of the Test

An anti-human IL-1 alphacoating antibody is adsorbed onto microwells.

Figure 1

![Coated Microwell](image_url)
Human IL-1 alpha present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human IL-1 alpha antibody is added and binds to human IL-1 alpha captured by the first antibody.

Figure 2

First Incubation

Following incubation unbound biotin-conjugated anti-human IL-1 alpha antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-1 alpha antibody.

Figure 3

Second Incubation

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 4

Third Incubation
A coloured product is formed in proportion to the amount of human IL-1 alpha present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IL-1 alpha standard dilutions and human IL-1 alpha sample concentration determined.

Figure 5

4. Reagents Provided

4.1 Reagents for human IL-1 alphaELISA BMS243/2CE (96 tests)

1 aluminium pouch with a Microwell Plate coated with polyclonal antibody to human IL-1 alpha
1 vial (70 µl) Biotin-Conjugate anti-human IL-1 alphapolyclonal antibody
1 vial (150 µl) Streptavidin-HRP
2 vials human IL-1 alpha Standard lyophilized, 200 pg/ml upon reconstitution
1 vial (12 ml) Sample Diluent

Please note: In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Sample Diluent vial. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.
1 vial (5 ml) Assay Buffer Concentrate 20x (PBS with 1% Tween 20, 10% BSA)
1 bottle (50 ml) Wash Buffer Concentrate 20x (PBS with 1% Tween 20)
1 vial (15 ml) Substrate Solution (tetramethyl-benzidine)
1 vial (15 ml) Stop Solution (1M Phosphoric acid)

4 Adhesive Films

4.2 Reagents for human IL-1 alphaELISA BMS243/2TENCE (10x96 tests)

10 aluminium pouches with a Microwell Plate coated with polyclonal antibody to human IL-1 alpha
10 vials (70 µl) Biotin-Conjugate anti-human IL-1 alphapolyclonal antibody
10 vials (150 µl) Streptavidin-HRP
10 human IL-1 alpha Standard lyophilized, 200 pg/ml upon reconstitution
7 vials (12 ml) Sample Diluent

Please note: In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Sample Diluent vial. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.
2 vials (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20, 10% BSA)
5 bottles (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)
10 vials (15 ml) **Substrate Solution** (tetramethyl-benzidine)
1 vial (100 ml) **Stop Solution** (1M Phosphoric acid)
20 **Adhesive Films**

5. **Storage Instructions – ELISA Kit**

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6. **Specimen Collection and Storage Instructions**

Cell culture supernatant, serum, plasma (EDTA, citrate, heparin) and urine were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible “**Hook Effect**” due to high sample concentrations (see chapter 11).

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human IL-1 alpha. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13.5).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7. **Materials Required But Not Provided**

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis
8. Precautions for Use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for in vitro diagnostic use and are not for use in therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9. Preparation of Reagents

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

9.1. Wash Buffer (1x)

Pour entire contents (50 ml) of the Wash Buffer Concentrate (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2°C to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
Wash Buffer (1x) may also be prepared as needed according to the following table:

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Wash Buffer Concentrate (20x) (ml)</th>
<th>Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>25</td>
<td>475</td>
</tr>
<tr>
<td>1 - 12</td>
<td>50</td>
<td>950</td>
</tr>
</tbody>
</table>

9.2. Assay Buffer (1x)

Pour the entire contents (5 ml) of the Assay Buffer Concentrate (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.

Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Assay Buffer Concentrate (20x) (ml)</th>
<th>Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>2.5</td>
<td>47.5</td>
</tr>
<tr>
<td>1 - 12</td>
<td>5.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

9.3. Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Biotin-Conjugate (ml)</th>
<th>Assay Buffer (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>2.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>

9.4. Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:200 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Streptavidin-HRP (ml)</th>
<th>Assay Buffer (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>5.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>11.94</td>
</tr>
</tbody>
</table>

9.5. Human IL-1 alpha Standard

Reconstitute human IL-1 alpha Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 200 pg/ml).

Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

After usage remaining standard cannot be stored and has to be discarded.
**Standard dilutions** can be prepared directly on the microwell plate (see 10.c) or alternatively in tubes (see 9.5.1).

### 9.5.1. External Standard Dilution

Label 7 tubes, one for each standard point.

S1, S2, S3, S4, S5, S6, S7

Then prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µl of Sample Diluent into each tube.

Pipette 225 µl of reconstituted standard (concentration of standard = 200 pg/ml) into the first tube, labelled S1, and mix (concentration of standard 1 = 100 pg/ml).

Pipette 225 µl of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.

Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6). Sample Diluent serves as blank.

Figure 6

![Transfer 225 µl](image)

**10. Test Protocol**

1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

2. Wash the microwell strips twice with approximately 400 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry.**

3. Standard **dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 9.5.1):

   Add 100 µl of Sample Diluent in duplicate to all **standard wells**. Pipette 100 µl of prepared **standard** (see Preparation of Standard 9.5, concentration = 200 pg/ml) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 100 pg/ml), and transfer 100 µl to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human IL-1 alpha standard dilutions ranging from 100.0 to 1.6 pg /ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.
In case of an external standard dilution (see 9.5.1), pipette 100 µl of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

Table 1
Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard 1 (100.0 pg/ml)</td>
<td>Standard 1 (100.0 pg/ml)</td>
<td>Sample 1</td>
<td>Sample 1</td>
</tr>
<tr>
<td>B</td>
<td>Standard 2 (50.0 pg/ml)</td>
<td>Standard 2 (50.0 pg/ml)</td>
<td>Sample 2</td>
<td>Sample 2</td>
</tr>
<tr>
<td>C</td>
<td>Standard 3 (25.0 pg/ml)</td>
<td>Standard 3 (25.0 pg/ml)</td>
<td>Sample 3</td>
<td>Sample 3</td>
</tr>
<tr>
<td>D</td>
<td>Standard 4 (12.5 pg/ml)</td>
<td>Standard 4 (12.5 pg/ml)</td>
<td>Sample 4</td>
<td>Sample 4</td>
</tr>
<tr>
<td>E</td>
<td>Standard 5 (6.3 pg/ml)</td>
<td>Standard 5 (6.3 pg/ml)</td>
<td>Sample 5</td>
<td>Sample 5</td>
</tr>
<tr>
<td>F</td>
<td>Standard 6 (3.1 pg/ml)</td>
<td>Standard 6 (3.1 pg/ml)</td>
<td>Sample 6</td>
<td>Sample 6</td>
</tr>
<tr>
<td>G</td>
<td>Standard 7 (1.6 pg/ml)</td>
<td>Standard 7 (1.6 pg/ml)</td>
<td>Sample 7</td>
<td>Sample 7</td>
</tr>
<tr>
<td>H</td>
<td>Blank</td>
<td>Blank</td>
<td>Sample 8</td>
<td>Sample 8</td>
</tr>
</tbody>
</table>

4. Add 100 µl of Sample Diluent in duplicate to the blank wells.
5. Add 50 µl of Sample Diluent to the sample wells.
6. Add 50 µl of each sample in duplicate to the sample wells.
7. Prepare Biotin-Conjugate (see Preparation of Biotin-Conjugate 9.3).
8. Add 50 µl of Biotin-Conjugate to all wells.
9. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 400 rpm.

10. Prepare **Streptavidin-HRP** (refer to Preparation of Streptavidin-HRP 9.4).

11. Remove adhesive film and empty wells. **Wash** microwell strips 4 times according to point b. of the test protocol. Proceed immediately to the next step.

12. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

13. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 200 rpm.

14. Remove adhesive film and empty wells. **Wash** microwell strips 4 times according to point b. of the test protocol. Proceed immediately to the next step.

15. Pipette 100 µl of **TMB Substrate Solution** to all wells.

16. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

   **The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.**

   It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

17. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

18. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

   **Note:** In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

### 11. Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-1 alpha concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

- To determine the concentration of circulating human IL-1 alpha for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IL-1 alpha concentration.

- If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µl sample + 50 µl Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

- **Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human IL-1 alphalevels (Hook Effect). Such samples require further external predilution according to expected human IL-1 alpahavalue with Sample Diluent in order to precisely quantitate the actual human IL-1 alpahalevel.**

- It is suggested that each testing facility establishes a control sample of known human IL-1 alpahavaleucncentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
● A representative standard curve is shown in Figure 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 8
Representative standard curve for human IL-1 alphaELISA. Human IL-1 alpha was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.
Table 2
Typical data using the human IL-1 alpha ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

<table>
<thead>
<tr>
<th>Standard</th>
<th>Human IL-1 alpha Concentration (pg/ml)</th>
<th>O.D. at 450 nm Mean</th>
<th>O.D. at 450 nm</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.0</td>
<td>2.206</td>
<td>2.203</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50.0</td>
<td>1.137</td>
<td>1.170</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.202</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25.0</td>
<td>0.619</td>
<td>0.608</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.597</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12.5</td>
<td>0.348</td>
<td>0.341</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.334</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.3</td>
<td>0.205</td>
<td>0.207</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.208</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3.1</td>
<td>0.167</td>
<td>0.163</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.159</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.6</td>
<td>0.108</td>
<td>0.106</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>0</td>
<td>0.069</td>
<td>0.070</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.070</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12. Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
13. Performance Characteristics

13.1. Sensitivity
The limit of detection of human IL-1 alpha defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 1.1 pg/ml (mean of 6 independent assays).

13.2. Reproducibility

13.2.1. Intra-assay
Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human IL-1 alpha. 2 standard curves were run on each plate. The calculated overall intra-assay coefficient of variation was < 5.4%.

13.2.2. Inter-assay
Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human IL-1 alpha. 2 standard curves were run on each plate. The calculated overall inter-assay coefficient of variation was < 10%.

13.3. Spike Recovery
The spike recovery was evaluated by spiking 4 levels of human IL-1 alpha into different samples. Recoveries were determined in 3 independent experiments with 4 replicates each. The amount of endogenous human IL-1 alpha in unspiked serum was subtracted from the spike values. The overall mean recovery was 92%.

13.4. Dilution Parallelism
Serum samples with different levels of human IL-1 alpha were analysed at serial 2 fold dilutions with 4 replicates each. The overall mean recovery was 99%.

13.5. Sample Stability

13.5.1. Freeze-Thaw Stability
Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human IL-1 alpha levels determined. There was no significant loss of human IL-1 alpha immunoreactivity detected by freezing and thawing.

13.5.2. Storage Stability
Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human IL-1 alpha level determined after 24 h. There was no significant loss of human IL-1 alpha immunoreactivity detected during storage at -20°C and 2-8°C.

A significant loss of human IL-1 alpha immunoreactivity (50%) was detected during storage at RT and 37°C after 24 h.

13.6. Specificity
The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human IL-1 alphapositive serum. There was no cross reactivity or interference detected.
13.7. Expected Values

Panels of 40 serum as well as EDTA, citrate and heparin plasma samples from randomly selected apparently healthy donors (males and females) were tested for human IL-1 alpha (see Table 3). The levels measured may vary with the sample collection used.

For detected human IL-1 alpha levels see Table 3.

Table 3

<table>
<thead>
<tr>
<th>Sample Matrix</th>
<th>Number of Samples Evaluated</th>
<th>Range (pg/ml)</th>
<th>% Detectable</th>
<th>Mean of Detectable pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>40</td>
<td>nd *- 3.9</td>
<td>2.5</td>
<td>--</td>
</tr>
<tr>
<td>Plasma (EDTA)</td>
<td>40</td>
<td>nd *- 10.5</td>
<td>5.0</td>
<td>9.1</td>
</tr>
<tr>
<td>Plasma (Citrate)</td>
<td>40</td>
<td>nd *</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Plasma (Heparin)</td>
<td>40</td>
<td>nd *- 51.7</td>
<td>17.5</td>
<td>13.7</td>
</tr>
</tbody>
</table>

* n.d. = non-detectable, samples measured below the lowest standard point are considered to be non-detectable.

14. Reagent Preparation Summary

14.1. Wash Buffer (1x)

Add Wash Buffer Concentrate 20x (50 ml) to 950 ml distilled water.

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Wash Buffer Concentrate (ml)</th>
<th>Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>25</td>
<td>475</td>
</tr>
<tr>
<td>1 - 12</td>
<td>50</td>
<td>950</td>
</tr>
</tbody>
</table>

14.2. Assay Buffer (1x)

Add Assay Buffer Concentrate 20x (5 ml) to 95 ml distilled water.

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Assay Buffer Concentrate (ml)</th>
<th>Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>2.5</td>
<td>47.5</td>
</tr>
<tr>
<td>1 - 12</td>
<td>5.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

14.3. Biotin-Conjugate

Make a 1:100 dilution of Biotin-Conjugate in Assay Buffer (1x):

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Biotin-Conjugate (ml)</th>
<th>Assay Buffer (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>2.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>
14.4. Streptavidin-HRP

Make a 1:200 dilution of Streptavidin-HRP in Assay Buffer (1x):

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Streptavidin-HRP (ml)</th>
<th>Assay Buffer (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>5.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>11.94</td>
</tr>
</tbody>
</table>

14.5. Human IL-1 alpha Standard

Reconstitute lyophilized human IL-1 alpha standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

15. Test Protocol Summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µl Sample Diluent, in duplicate, to all standard wells. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 100 µl from well to well. Discard 100 µl from the last wells. Alternatively external standard dilution in tubes (see 9.5.1): Pipette 100 µl of these standard dilutions in the microwell strips.
4. Add 100 µl Sample Diluent, in duplicate, to the blank wells.
5. Add 50 µl Sample Diluent to sample wells.
6. Add 50 µl sample in duplicate, to designated sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50 µl Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 4 times with Wash Buffer.
12. Add 100 µl diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C).
14. Empty and wash microwell strips 4 times with Wash Buffer.
15. Add 100 µl of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
17. Add 100 µl Stop Solution to all wells.
18. Blank microwell reader and measure colour intensity at 450 nm.

Note: If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µl sample + 50 µl Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
1. Mitgelieferte Reagenzien

1.1. Mitgelieferte Reagenzien für human IL-1 alphaELISA

BMS243/2CE (96 Tests)

1. Aluminiumbeutel mit Mikrotiterplatte, beschichtet mit Antikörper (polyklonal) gegen human IL-1 alpha
2. Fläschchen (70 µl) Biotin-Konjugat, polyklonaler anti-human IL-1 alpha-Antikörper
3. Fläschchen (150 µl) Streptavidin-HRP
4. Fläschchen human IL-1 alpha-Standard, lyophilisiert, 200 pg/ml nach Rekonstitution
5. Fläschchen (12 ml) Verdünnungslösung


1. Fläschchen (5 ml) Probenpufferkonzentrat 20x (PBS mit 1% Tween 20, 10% BSA)
2. Flasche (50 ml) Waschpufferkonzentrat 20x (PBS mit 1% Tween 20)
3. Fläschchen (15 ml) Substratlösung (Tetramethylbenzidin)
4. Fläschchen (15 ml) Stopplösung (1 M Phosphorsäure)

2. Mitgelieferte Reagenzien für human IL-1 alphaELISA

BMS243/2TENCE (10x96 Tests)

10. Aluminiumbeutel mit Mikrotiterplatte, beschichtet mit Antikörper (polyklonal) gegen human IL-1 alpha
10. Fläschchen (70 µl) Biotin-Konjugat, polyklonaler anti-human IL-1 alpha-Antikörper
10. Fläschchen (150 µl) Streptavidin-HRP
10. Fläschchen human IL-1 alpha-Standard, lyophilisiert, 200 pg/ml nach Rekonstitution
7. Fläschchen (12 ml) Verdünnungslösung


2. Fläschchen (5 ml) Probenpufferkonzentrat 20x (PBS mit 1% Tween 20, 10% BSA)
5. Flaschen (50 ml) Waschpufferkonzentrat 20x (PBS mit 1% Tween 20)
10. Fläschchen (15 ml) Substratlösung (Tetramethylbenzidin)
1. Fläschchen (100 ml) Stopplösung (1 M Phosphorsäure)

20. Klebefolien
2. Lagerhinweise


3. Sicherheitsvorkehrungen für den Gebrauch

- Die Reagenzien sind ausschließlich für Diagnosezwecke bestimmt und nicht für den Einsatz bei Therapien.
- Reagenzien aus verschiedenen Chargen oder anderer Herkunft nicht mischen oder untereinander austauschen.
- Verwenden Sie die Kitreagenzien nicht nach dem Ablaufdatum (siehe Etikett).
- Setzen Sie die Kitreagenzien während der Lagerung oder Inkubation keiner starken Lichteinstrahlung aus.
- Nicht mit dem Mund pipettieren.
- Im Bereich, in denen mit Kitreagenzien oder Proben hantiert wird, nicht essen, trinken oder rauchen.
- Vermeiden Sie den Kontakt der Haut/Schleimhäute mit Kitreagenzien/Proben.
- Vermeiden Sie Verspritzen von Flüssigkeit oder Bildung von Aerosolen.
- Vermeiden Sie Kontamination mit Mikroben oder Kreuzkontamination der Reagenzien oder Proben, die den Test ungültig machen könnten, verwenden Sie Einwegpipettenspitzen und/oder Einwegpipetten.
- Verwenden Sie saubere, geeignete Reagenzgefäße für das Dispensieren von Konjugat und Substratreagenzien.
- Vermeiden Sie Kontakt mit Säuren, da dadurch Konjugate inaktiviert werden.
- Für die Reagensherstellung muss destilliertes oder entionisiertes Wasser verwendet werden.
- Die Substratlösung muss vor der Verwendung auf Raumtemperatur gebracht werden.
- Dekontaminieren und entsorgen Sie Proben sowie alle möglicherweise kontaminierten Materialien so, als ob sie Infektionserreger enthalten könnten. Die bevorzugte Dekontaminationsmethode ist Autoklavieren für mind. eine Stunde bei 121,5°C.
- Flüssige Abfälle, die kein Säure enthalten, sowie neutralisierte Abfälle werden zur Dekontamination mit Natrium Hypochlorit versetzt (Endkonzentration von Natrium Hypochlorit 1.0%). Nach 30 min ist eine effektive Dekontamination erreicht. Flüssige Abfälle, die Säure enthalten, müssen vor der Dekontamination neutralisiert werden.
4. Vorbereitung der Reagenzien

Bringen Sie die Pufferkonzentrate auf Raumtemperatur und stellen Sie die Verdünnungen vor Beginn des Tests her. Sollten sich in den Pufferkonzentraten Kristalle gebildet haben, erwärmen Sie diese vorsichtig bis zur vollständigen Auflösung der Kristalle.

4.1. Waschpuffer (1x)

Leeren Sie den gesamten Inhalt (50 ml) des Waschpufferkonzentrats (20x) in einen sauberen 1000-ml-Messzylinder. Füllen Sie mit destilliertem oder entionisiertem Wasser auf, bis ein Endvolumen von 1000 ml erreicht ist. Mischen Sie vorsichtig um Schäumen zu vermeiden.

Füllen Sie in eine saubere Waschflasche um und lagern Sie den Waschpuffer (1x) bei 2° bis 25°C lagern. Bitte beachten Sie, dass dieser 30 Tage haltbar ist.

Der benötigte Waschpuffer (1x) kann auch entsprechend der untenstehenden Tabelle hergestellt werden:

<table>
<thead>
<tr>
<th>Anzahl der Streifen</th>
<th>Waschpufferkonzentrat (20x) (ml)</th>
<th>Destilliertes Wasser (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>25</td>
<td>475</td>
</tr>
<tr>
<td>1 - 12</td>
<td>50</td>
<td>950</td>
</tr>
</tbody>
</table>

4.2. Probenpuffer (1x)

Leeren Sie den gesamten Inhalt (5 ml) des Probenpufferkonzentrates (20x) in einen sauberen 100-ml-Messzylinder. Füllen Sie mit destilliertem oder entionisiertem Wasser auf, bis ein Endvolumen von 100 ml erreicht ist. Mischen Sie vorsichtig um Schäumen zu vermeiden.

Probenpuffer (1x) bei 2° bis 8°C lagern. Bitte beachten Sie, dass der Probenpuffer (1x) 30 Tage haltbar ist.

Der benötigte Probenpuffer (1x) kann auch entsprechend der untenstehenden Tabelle hergestellt werden:

<table>
<thead>
<tr>
<th>Anzahl der Streifen</th>
<th>Probenpufferkonzentrat (20x) (ml)</th>
<th>Destilliertes Wasser (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>2.5</td>
<td>47.5</td>
</tr>
<tr>
<td>1 - 12</td>
<td>5.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

4.3. Biotin-Konjugat

Bitte beachten Sie, dass die Biotin-Konjugatlösung nach der Verdünnung nur 30 Minuten haltbar ist.

Stellen Sie eine 1:100 Verdünnung der konzentrierten Biotin-Konjugatlösung in Probenpuffer (1x) in einem sauberen Gefäß entsprechend der untenstehenden Tabelle her.

<table>
<thead>
<tr>
<th>Anzahl der Streifen</th>
<th>Biotin-Konjugat (ml)</th>
<th>Probenpuffer (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>2.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>

4.4. Streptavidin-HRP

Bitte beachten Sie, dass die Streptavidin-HRP-Lösung nach der Verdünnung nur 30 Minuten haltbar ist. Stellen Sie eine 1:200 Verdünnung der konzentrierten Streptavidin-HRP-Lösung in Probenpuffer (1x) in einem sauberen Gefäß entsprechend der untenstehenden Tabelle her.
<table>
<thead>
<tr>
<th>Anzahl der Streifen</th>
<th>Streptavidin-HRP (ml)</th>
<th>Probenpuffer (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>5.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>11.94</td>
</tr>
</tbody>
</table>

**4.5. Human IL-1 alpha-Standard**


Den rekonstituierten Standard nach 10-30 min verdünnen und davor gut mischen. Der Standard muß sofort nach Rekonstitution verwendet und kann nicht gelagert werden.

Die **Standardverdünnungen** können direkt auf den Mikrotiterplatten (siehe 5.c) oder in Reaktionsgefäßen (siehe 4.5.1) hergestellt werden.

**4.5.1. Externe Standardverdünnung**

Beschreiben Sie 7 Gefäße, jedes für einen Standardpunkt wie folgt: S1, S2, S3, S4, S5, S6, S7

Stellen Sie eine 1:2 Verdünnungsreihe für die Standardkurve her: Pipettieren Sie in jedes Gefäß 225 µl der Verdünnungslösung.

Pipettieren Sie 225 µl des rekonstituierten Standards (Konzentration des Standards = 200 pg/ml) in das erste Gefäß mit der Beschriftung S1 und mischen Sie (Konzentration des Standard 1 = 100 pg/ml).

Pipettieren Sie 225 µl dieser Verdünnung in das zweite Gefäß (mit der Beschriftung S2) und mischen Sie sorgfältig vor dem nächsten Verdünnungsschritt.

Wiederholen Sie diese Verdünnungsschritte 5x. Die so hergestellte Verdünnungsreihe dient zur Erstellung der Standardkurve (siehe Figure 9).

Verdünnungslösung dient als Blindwert.

Figure 9
5. Testprotokoll


3. **Standardverdünnung auf der Mikrotiterplatte** (Wahlweise können die Standardverdünnungen auch in Reaktionsgefäßen hergestellt werden – siehe 4.5.1)

   Pipettieren Sie 100 µl Verdünnungslösung in alle **Standardvertiefungen**. Pipettieren Sie 100 µl des rekonstituierten **Standards** (siehe Herstellung des Standards 4.5. Konzentration des Standards = 200.0 pg/ml) in die Vertiefungen A1 und A2 (Doppelbestimmung, siehe Table 4). Mischen Sie den Inhalt der Vertiefungen A1 und A2 durch wiederholtes Aufsaugen und Zugeben gut durch (Konzentration des Standards S1 = 100.0 pg/ml) und transferieren Sie 100 µl in die Probenvertiefungen B1 und B2 (siehe Figure 10). Achten Sie darauf, die Oberfläche der Vertiefungen nicht zu zerkratzen. Wiederholen Sie diese Verdünnungsschritte 5 x, wodurch zwei human IL-1 alpha Verdünnungsreihen mit den Konzentrationen von 100.0 bis 1.6 pg/ml hergestellt werden. Verwerfen Sie 100 µl aus den letzten Standardvertiefungen (G1/2). Die so hergestellten Verdünnungsreihen dienen zur Erstellung der Standardkurve.

![Figure 10](image-url)

Falls sie eine **externe Standardverdünnungsreihe** erstellen (siehe 4.5.1), pipettieren Sie 100 µl der Standardverdünnungen (S1 – S7) in die Standardvertiefungen (entsprechend Table 4).
Table 4
Diagramm mit Beispiel für die Anordnung von Blindwert, Standards und Proben in den Mikrowellstreifen:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard 1 (100.0 pg/ml)</td>
<td>Standard 1 (100.0 pg/ml)</td>
<td>Probe 1</td>
<td>Probe 1</td>
</tr>
<tr>
<td>B</td>
<td>Standard 2 (50.0 pg/ml)</td>
<td>Standard 2 (50.0 pg/ml)</td>
<td>Probe 2</td>
<td>Probe 2</td>
</tr>
<tr>
<td>C</td>
<td>Standard 3 (25.0 pg/ml)</td>
<td>Standard 3 (25.0 pg/ml)</td>
<td>Probe 3</td>
<td>Probe 3</td>
</tr>
<tr>
<td>D</td>
<td>Standard 4 (12.5 pg/ml)</td>
<td>Standard 4 (12.5 pg/ml)</td>
<td>Probe 4</td>
<td>Probe 4</td>
</tr>
<tr>
<td>E</td>
<td>Standard 5 (6.3 pg/ml)</td>
<td>Standard 5 (6.3 pg/ml)</td>
<td>Probe 5</td>
<td>Probe 5</td>
</tr>
<tr>
<td>F</td>
<td>Standard 6 (3.1 pg/ml)</td>
<td>Standard 6 (3.1 pg/ml)</td>
<td>Probe 6</td>
<td>Probe 6</td>
</tr>
<tr>
<td>G</td>
<td>Standard 7 (1.6 pg/ml)</td>
<td>Standard 7 (1.6 pg/ml)</td>
<td>Probe 7</td>
<td>Probe 7</td>
</tr>
<tr>
<td>H</td>
<td>Blindwert</td>
<td>Blindwert</td>
<td>Probe 8</td>
<td>Probe 8</td>
</tr>
</tbody>
</table>

4. Pipettieren Sie in alle Blindwertvertiefungen (Doppelbestimmung), 100 µl Verdünnungslösung.
5. Pipettieren Sie in alle Probenvertiefungen 50 µl Verdünnungslösung.
6. Pipettieren Sie je 50 µl von jeder Probe (Doppelbestimmung) in die Probenvertiefungen und mischen Sie den Inhalt durch.
7. Stellen Sie Biotin-Konjugat (siehe: Vorbereitung der Reagenzien Biotin-Konjugat 4.3) her.
8. Pipettieren Sie in alle Vertiefungen, einschließlich der Blindwertvertiefungen 50 µl Biotin-Konjugat.
9. Mit einer Klebefolie abdecken und bei Raumtemperatur (18° bis 25°C) für 2 Stunden inkubieren, wenn möglich auf einem Schüttler bei 200 U/min.
10. Stellen Sie Streptavidin-HRP (siehe: Vorbereitung der Reagenzien Streptavidin-HRP 4.4) her.
12. Pipettieren Sie in alle Vertiefungen, einschließlich der Blindwertvertiefungen 100 µl Streptavidin-HRP.
13. Mit einer Klebefolie abdecken und bei Raumtemperatur (18° bis 25°C) für 1 Stunde inkubieren, wenn möglich auf einem Schüttler bei 200 U/min.
15. Pipettieren Sie in alle Vertiefungen, einschließlich der Blindwertvertiefungen, 100 µl TMB-Substratlösung.
16. Inkubieren Sie die Mikrowellstreifen bei Raumtemperatur (18° bis 25°C) für ca. 10 Minuten. Vermeiden Sie direkte, starke Lichteinstrahlung.

Die Farbentwicklung innerhalb der einzelnen Vertiefungen muss beobachtet und die Substratreaktion gestoppt werden (siehe nächster Protokollpunkt), bevor die gefärbten Vertiefungen nicht mehr richtig gemessen werden können. Die optimale Inkubationszeit für die Farbentwicklung muß bei jedem Versuch neu bestimmt werden.
Es wird empfohlen, die Stopplösung zuzugeben, wenn der höchste Standardpunkt eine dunkelblaue Farbe angenommen hat.
Alternativ kann die Farbentwicklung auch mit einem Photometer bei 620 nm verfolgt werden. Die Substratreaktion sollte gestoppt werden, wenn der höchste Standardpunkt eine OD von 0.9 - 0.95 erreicht.


Die Proben wurden im Zuge der Testdurchführung 1:2 verdünnt. Daher muß der aus der Standardkurve berechnete Wert mit dem Verdünnungsfaktor multipliziert werden (x 2).

Note: Falls die Platte während der Inkubation nicht geschüttelt wurde, können die erreichten OD Werte niedriger als die unten angeführten sein. Die Ergebnisse sind trotzdem gültig.
INFORMACIÓN Y MANUAL DEL PRODUCTO (Espanol)

1. Reactivos Suministrados

1.1. Reactivos Suministrados para human IL-1 alphaELISA
BMS243/2CE (96 tests)

1 bolsa de aluminio con una placa de micropocillos recubiertos con anticuerpos policlonales anti-human IL-1 alpha
1 vial (70 µl) con conjugado de biotina (anticuerpos policlonales anti-human IL-1 alpha)
1 vial (150 µl) con estreptavidina-HRP
2 viales con Estándard human IL-1 alphaliofilizado , 200 pg/ml tras la reconstitución
1 vial (12 ml) de diluyente de muestra

Nota: En algunos (muy raros) casos un precipitado insoluble de proteína estabilizante ha sido observado en el vial. Este precipitado no interfiere de ninguna manera con el desarrollo del test y puede ser, por tanto, ignorado.

1 vial (5 ml) de concentrado de tampón de ensayo 20x (PBS con Tween 20 al 1% y BSA al 10%)
1 frasco (50 ml) de concentrado de tampón de lavado 20x (PBS con Tween 20 al 1%)
1 vial (15 ml) de solución de sustrato (tetrametil-bencidina)
1 vial (15 ml) de solución de parada (ácido fosfórico 1M)
4 tapas para placas, adhesives

1.2. Reactivos Suministrados para human IL-1 alphaELISA
BMS243/2TENCE (10x96 tests)

10 bolsas de aluminio con una placa de micropocillos recubiertos con anticuerpos policlonales anti-human IL-1 alpha
10 viales (70 µl) con conjugado de biotina (anticuerpos policlonales anti-human IL-1 alpha)
10 viales (150 µl) con estreptavidina-HRP
10 viales con Estándard human IL-1 alphaliofilizado , 200 pg/ml tras la reconstitución
7 viales (12 ml) de diluyente de muestra

Nota: En algunos (muy raros) casos un precipitado insoluble de proteína estabilizante ha sido observado en los viales. Este precipitado no interfiere de ninguna manera con el desarrollo del test y puede ser, por tanto, ignorado.

2 viales (5 ml) de concentrado de tampón de ensayo 20x (PBS con Tween 20 al 1% y BSA al 10%)
5 frascos (50 ml) de concentrado de tampón de lavado 20x (PBS con Tween 20 al 1%)
10 viales (15 ml) de solución de sustrato (tetrametil-bencidina)
1 vial (100 ml) de solución de parada (ácido fosfórico 1M)
20 tapas para placas, adhesives
2. Instrucciones de Conservación

Conservar los reactivos del kit a una temperatura comprendida entre 2 y 8°C. Inmediatamente después de utilizarlos deberá volver a conservar los reactivos a dicha temperatura (2° to 8°C). En las etiquetas figuran las fechas de caducidad del kit y de los reactivos.

Sólo se podrá garantizar la fecha de caducidad de los componentes del kit si se conservan adecuadamente y, en caso de uso reiterado de un mismo componente, si el reactivo no queda contaminado en la primera manipulación.

3. Precauciones de uso

- Todos los productos químicos deben considerarse potencialmente peligrosos. Por tanto, recomendamos que este producto sea manipulado únicamente por aquellas personas que hayan sido entrenadas en técnicas de laboratorio y que sea usado de acuerdo con los principios de buenas prácticas de laboratorio. Se debe llevar ropa de protección apropiada como puedan ser las batas de laboratorio, gafas de seguridad y guantes. Se debe trabajar con cuidado para evitar cualquier contacto con piel y ojos. En el caso de que tenga lugar un contacto con piel o ojos, proceder de forma inmediata a lavar la parte afectada con abundante agua. Véase la(s) hoja(s) de seguridad y/o declaraciones de seguridad para recomendaciones específicas.
- Los reactivos están destinados para un uso en diagnóstico in vitro y no se deben usar en procedimientos terapéuticos.
- No mezclar o sustituir los reactivos por los equivalentes de otros lotes u otras fuentes.
- No usar reactivos caducados.
- No exponer los reactivos del kit a una luz intensa durante su almacenamiento o incubación.
- No pipetear con la boca.
- No se recomienda comer o fumar en las zonas donde se manipulen muestras o reactivos.
- Evitar el contacto de los reactivos del kit o de las muestras con piel o mucosas.
- Se recomienda el uso de guantes desechables de goma o látex durante la manipulación de las muestras y reactivos.
- Evitar el contacto de la solución de sustrato con agentes oxidantes y metales.
- Evitar salpicaduras y la generación de aerosoles.
- Con el propósito de evitar una contaminación microbiológica o contaminaciones cruzadas de reactivos y muestras que puedan invalidar el test se recomienda el uso de pipetas y/o puntas de pipetas de un solo uso.
- Usar recipientes limpios y específicos de reactivos para la dispensación de reactivos de sustrato.
- La exposición a los ácidos inactiva el conjugado.
- Se debe usar agua destilada o desionizada en la preparación de los reactivos.
- La solución de sustrato debe de estar a temperatura ambiente antes de su uso.
- Descontaminar y disponer las muestras y todos los materiales potencialmente contaminados como si pudieran contener agentes infecciosos. El método preferente de descontaminación es un autoclavado durante un mínimo de 1 hora a 121.5°C.
- Los residuos líquidos que no contengan ácido y los residuos neutralizados pueden ser mezclados con hipoclorito sódico en volúmenes tales que la mezcla final contenga 1.0% de hipoclorito sódico. Dejar actuar durante 30 minutos para una efectiva descontaminación. Los residuos líquidos que contengan ácido deben ser neutralizados previamente a la adición de hipoclorito sódico.
4. Preparación de los Reactivos

Los tampónes concentrados debe de alcanzar la temperatura ambiente y ser diluidos antes de iniciar el procedimiento del test. Si en el concentrado de tampónes concentrados se han formado cristales, caliente suavemente hasta su completa disolución.

4.1. Tampón de Lavado (1x)

Vierta todo el contenido (50 ml) del concentrado de tampón de lavado (20x) en un matraz aforado de 1000 ml limpio. Enrase en matraz con agua destilada o desionizada. Mezcle suavemente para evitar la formación de espuma.

Transfiera la solución a un frasco de lavado limpio y consérvela a una temperatura entre 2°C y 25°C. El tampón de lavado permanece estable durante 30 días.

En función de la cantidad que vaya a necesitar, prepare el tampón de lavado de acuerdo a la siguiente tabla:

<table>
<thead>
<tr>
<th>Número de tiras</th>
<th>Tampón de lavado (20x) (ml)</th>
<th>Agua destilada (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 6</td>
<td>25</td>
<td>475</td>
</tr>
<tr>
<td>1 - 12</td>
<td>50</td>
<td>950</td>
</tr>
</tbody>
</table>

4.2. Tampón de ensayo (1x)

Vierta todo el contenido (5 ml) del concentrado de tampón de ensayo (20x) en un matraz aforado de 100 ml limpio. Enrase en matraz con agua destilada o desionizada. Mezcle suavemente para evitar la formación de espuma.

Conserve la solución a una temperatura de entre 2°C y 8°C. El tampón de trabajo permanece estable durante 30 días.

En función de la cantidad que vaya a necesitar, prepare el tampón de ensayo de acuerdo a la siguiente tabla:

<table>
<thead>
<tr>
<th>Número de tiras</th>
<th>Tampón de ensayo (20x) (ml)</th>
<th>Agua destilada (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>2.5</td>
<td>47.5</td>
</tr>
<tr>
<td>1 - 12</td>
<td>5.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

4.3. Conjugado de biotina

Se utilizará el conjugado de biotina antes de transcurridos 30 minutos desde su dilución.

Justo antes de utilizar el conjugado de biotina, se debe diluirlo con tampón de ensayo (1x) en un tubo de ensayo de plástico limpio, en una proporción de 1:100.

En función de la cantidad que vaya a necesitar, prepare el conjugado de biotina de acuerdo a la siguiente tabla:

<table>
<thead>
<tr>
<th>Número de tiras</th>
<th>Conjugado de biotina (ml)</th>
<th>tampón de ensayo (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>2.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>

4.4. Estreptavidina-HRP

Se utilizará el estreptavidina-HRP antes de transcurridos 30 minutos desde su dilución.

Se debe diluir la estreptavidina-HRP con tampón de ensayo (1x) en un tubo de ensayo de plástico limpio, en una proporción de 1:200.
En función de la cantidad que vaya a necesitar, prepare la estreptavidina-HRP de acuerdo a la siguiente tabla:

<table>
<thead>
<tr>
<th>Número de tiras</th>
<th>Estreptavidina-HRP (ml)</th>
<th>tampón de ensayo (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>5.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>11.94</td>
</tr>
</tbody>
</table>

4.5. Dilución estándar human IL-1 alpha

Reconstituir el **estándar human IL-1 alpha** con la adición de agua destilada. El volumen de reconstitución está indicado en la etiqueta del vial del estándar. Girar o mezclar cuidadosamente para garantizar una completa y homogénea solubilización (concentración del estándar reconstituido = 200 pg/ml).

Permitir que el estándar reconstituido se asiente durante 10-30 minutos. Mezclar bien previamente a realizar las diluciones.

Tras su uso los restos del estándar no pueden ser almacenados y deben ser descartados.

Las **diluciones estándar** pueden ser preparadas directamente en la placa multipilóbulo (véase 5.b) o alternativamente en tubos (véase 4.5.1).

**4.5.1. Dilución Estándar Externa**

Rotular 7 tubos, uno para cada punto de la curva estándar.

S1, S2, S3, S4, S5, S6, S7.

Acto seguido, preparar diluciones seriadas 1:2 para la curva estándar como se indica a continuación:

Pipetear 225 µl de diluyente de muestra a todos los tubos.

Pipetear 225 µl de estándar reconstituido (concentración del estándar = 200 pg/ml) en el primer tubo, etiquetado como S1, y mezclar (concentración del estándar 1 = 100 pg/ml).

Pipetear 225 µl de esta dilución en el segundo tubo, etiquetado como S2, y mezclar completamente antes de la siguiente transferencia.

Repetir la serie de diluciones 5 veces más de manera que se obtengan los diferentes puntos de la curva estándar (véase Figure 11).

Diluyente de muestra cumple como blanco.

**Figure 11**
5. Protocolo de Ensayo

1. Determine el número de tiras necesarias para analizar el número deseado de muestras y además añada las tiras para blancos y patrones (de color). Todas las muestras, estándares, blancos deben ser analizadas por duplicado. Retire del soporte las tiras sobrantes y consérvelas, junto con el desecante suministrado en una bolsa metalizada y cerrada herméticamente, a una temperatura de 2°-8° C. Coloque las tiras que contienen la curva de valoración en las posiciones A1/A2 a H1/H2 (véase la Table 5).

2. Lave 2 veces las tiras con aproximadamente 400 µl de tampón de lavado por cada pocillo, aspirando completamente el contenido de los pocillos entre cada lavado. Permitir que el tampón de lavado permanezca en los pocillos durante 10-15 segundos antes de su aspiración. Evite rayar la superficie de los pocillos. Tras el último lavado, golpee suavemente las tiras contra un papel absorbente o una toallita de papel para eliminar el exceso de tampón de lavado. Utilice las tiras inmediatamente después de lavadas o bien colóquelas boca abajo sobre un papel absorbente húmedo durante como máximo 15 minutos. No deje secar los pocillos.

3. **Dilución de los Estándares en la placa multipocillo** (Alternativamente, la dilución de los estándars puede ser preparada en tubos – véase 4.5.1) Añadir 100 µl de diluyente de muestra a todos los pocillos estándar. Pipetear 100 µl de estándar preparado (véase Preparación del Estándar 4.5, concentración = 200.0 pg/ml) por duplicado en los pocillos A1 y A2 (véase Table 5). Mezclar el contenido de los pocillos A1 y A2 por repetidas aspiraciones y expulsiones del contenido con la pipeta (concentración del estándar 1, S1 =100.0 pg/ml), y transferir 100 µl a los pocillos B1 y B2, respectivamente (véase Figure 12). Levar cuidado de no rascar la superficie interior de los micropocillos con la punta de la pipeta. Continuar este procedimiento 5 veces, formando dos filas de diluciones estándar del human IL-1 alpha ordenadas des de 100.0 a 1.6 pg/ml. Descartar 100 µl de los contenidos de los últimos micropocillos (G1, G2) usados.

Figure 12

En caso de **una dilución estándar externa** (véase 4.5.1), pipetear 100 µl de estas diluciones estándar (S1 - S7) en los pocillos correspondientes al estándar de acuerdo con la Table 5.

4. Añada 100 µl **diluyente de muestra** los **pocillos del blanco**, por duplicado.

5. Añada 50 µl de **diluyente de muestra** los **pocillos con muestras**.

6. Por duplicado, añada 50 µl de cada **muestra a los pocillos designados**.
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Estándar 1 (100.0 pg/ml)</td>
<td>Estándar 1 (100.0 pg/ml)</td>
<td>Muestra 1</td>
<td>Muestra 1</td>
</tr>
<tr>
<td>B</td>
<td>Estándar 2 (50.0 pg/ml)</td>
<td>Estándar 2 (50.0 pg/ml)</td>
<td>Muestra 2</td>
<td>Muestra 2</td>
</tr>
<tr>
<td>C</td>
<td>Estándar 3 (25.0 pg/ml)</td>
<td>Estándar 3 (25.0 pg/ml)</td>
<td>Muestra 3</td>
<td>Muestra 3</td>
</tr>
<tr>
<td>D</td>
<td>Estándar 4 (12.5 pg/ml)</td>
<td>Estándar 4 (12.5 pg/ml)</td>
<td>Muestra 4</td>
<td>Muestra 4</td>
</tr>
<tr>
<td>E</td>
<td>Estándar 5 (6.3 pg/ml)</td>
<td>Estándar 5 (6.3 pg/ml)</td>
<td>Muestra 5</td>
<td>Muestra 5</td>
</tr>
<tr>
<td>F</td>
<td>Estándar 6 (3.1 pg/ml)</td>
<td>Estándar 6 (3.1 pg/ml)</td>
<td>Muestra 6</td>
<td>Muestra 6</td>
</tr>
<tr>
<td>G</td>
<td>Estándar 7 (1.6 pg/ml)</td>
<td>Estándar 7 (1.6 pg/ml)</td>
<td>Muestra 7</td>
<td>Muestra 7</td>
</tr>
<tr>
<td>H</td>
<td>Blanco</td>
<td>Blanco</td>
<td>Muestra 8</td>
<td>Muestra 8</td>
</tr>
</tbody>
</table>

7. Prepare el conjugado de biotiona (véase la preparación de conjugado de biotiona 4.3)
8. Añada 50 µl el conjugado de biotiona a todos los pocillos.
9. Cubra la placa con una tapa e incúbela a temperatura ambiente (18°C - 25°C) durante 2 horas (en un agitador mecánico a 400 rpm, si es posible).
11. Retire la tapa y vacíe los pocillos. Lavar los micropocillos de las tiras 4 veces de acuerdo al punto b del protocolo del test. Proseguir inmediatamente después al próximo paso.
12. Añada 100 µl estreptavidina-HRP a todos los pocillos.
13. Cubra la placa con una tapa e incúbela a temperatura ambiente (18°C - 25°C) durante 1 hora (en un agitador mecánico a 400 rpm, si es posible)
15. Pipetee 100 µl de solución de sustrato TMB y viértalos en todos los pocillos, incluidos los del blanco.
16. Incube las tiras a temperatura ambiente (18°C - 25°C) durante aproximadamente 10 minutos. Evite la exposición directa a la luz intensa.

**Deben monitorizarse los valores DO de la placa para detener la reacción del sustrato (véase el siguiente punto de este protocolo) antes de que deje de ser posible registrar correctamente los pocillos positivos. La determinación del tiempo adecuado para el desarrollo del color, debe realizarse de forma individual para cada ensayo.**

Se recomienda añadir la solución de parada cuando el estándar más alto presente un color azul oscuro. Alternativamente el desarrollo de color puede ser monitorizado con un lector de placas de ELISA a 620 nm. La reacción del substrato debería ser parada cuando este estándar alcanze una DO entre 0.9 y 0.95.
17. Detenga la reacción enzimática pipeteando rápidamente 100 µl de solución de parada en cada pocillo, incluidos los del blanco. Es importante dispensar la solución de parada de forma rápida y uniforme en todos los pocillos para inactivar totalmente la enzima.

Los resultados deben leerse inmediatamente después de añadir la solución de parada o, como máximo, en el plazo de 1 hora si las tiras se conservan a una temperatura entre 2 - 8°C en un lugar oscuro.

18. Lea la absorbancia de cada pocillo en un espectrofotómetro utilizando 450 nm como longitud de onda principal (opcionalmente 620 nm como longitud de onda de referencia; los valores comprendidos entre 610 nm y 650 nm son aceptables). Utilizando los pocillos de blanco, haga el blanco del lector de placas de acuerdo con las instrucciones del fabricante. Determine la absorbancia de las muestras y de los human IL-1 alpha.

Las muestras han sido diluidas 1:2, por tanto la concentración leída a partir de la curva estándar debe ser multiplicada por el factor de dilución (x 2).

Note: En caso de incubar sin agitar, los valores de D.O. pueden ser inferiores a los indicados más abajo. De todas formas los resultados siguen siendo válidos.
INFORMATIONS SUR LE PRODUIT ET MANUEL (Français)

1. Réactifs Fournis

1.1. Reactifs pour ELISA de human IL-1 alphaBMS243/2CE (96 essais)

1. pochette en aluminium contenant une plaque de microtitration recouverte d’anticorps polyclonaux anti-human IL-1 alpha
2. flacon (70 µl) de conjugué biotine anti-human IL-1 alpha(anticorps polyclonaux anti-human IL-1 alpha)
3. flacon (150 µl) de streptavidine-HRP
4. flacons d’étalon human IL-1 alpha, lyophilisé, 200 pg/ml après reconstitution

Veuillez noter: Par rares occasions un precipité insoluble de proteines stabilisatrices a pu être observé dans la tube. Ce precipité n’interfère d’aucune manière avec le test et peut donc être ignoré.

1. flacon (5 ml) tampon d’essai concentré 20x (PBS avec Tween 20 1% et de la sérumalbumine bovine 10%)
2. flacon (50 ml) de tampon de lavage concentré 20 x (PBS avec du Tween 20 1 %)
3. flacon (15 ml) de solution de substrat (tétraméthyle-benzidine)
4. flacon (15 ml) de solution d’arrêt (acide phosphorique 1 M)

1.2. Reactifs pour ELISA de human IL-1 alphaBMS243/2TENCE (10x96 essais)

10. pochettes en aluminium contenant une plaque de microtitration recouverte d’anticorps polyclonaux anti-human IL-1 alpha
11. flacons (70 µl) de conjugué biotine anti-human IL-1 alpha(anticorps polyclonaux anti-human IL-1 alpha)
12. flacons (150 µl) de streptavidine-HRP
13. flacons d’étalon human IL-1 alpha, lyophilisé, 200 pg/ml après reconstitution

Veuillez noter: Par rares occasions un precipité insoluble de proteines stabilisatrices a pu être observé dans les tubes. Ce precipité n’interfère d’aucune manière avec le test et peut donc être ignoré.

2. flacons (5 ml) tampon d’essai concentré 20x (PBS avec Tween 20 1% et de la sérumalbumine bovine 10%)
5. flacons (50 ml) de tampon de lavage concentré 20 x (PBS avec du Tween 20 1 %)
10. flacons (15 ml) de solution de substrat (tétraméthyle-benzidine)
1. flacon (100 ml) de solution d’arrêt (acide phosphorique 1 M)
20. couvre-plaques adhésifs
2. Instruction de Stockage

Conserver les réactifs du kit entre 2° et 8°C. Immédiatement après l'utilisation, les réactifs doivent être rangés au frais (2° à 8°C). La date de péremption du kit est spécifiée sur les étiquettes.

Le délai de péremption du kit ne peut être garanti que si les composants sont conservés correctement et si, en cas d'utilisation répétée d'un composant, le réactif n'a pas été contaminé lors d'une première utilisation.

3. Préventions de Sécurité pour l’Usage

- Tout réactif doit être considéré comme potentiellement dangereux. Pour cela il est recommandé que ce produit est utilisé que par des personnes ayant une qualification de laboratoire et qu’il soit utilisé à l’avenant au code GLP. Une tenue correspondante comme des une blouse de travail, des lunettes protectrices et des gants de travail doivent-être portés. Evitez tous contactes de réactifs avec la peau ou les yeux. En cas de contact avec les yeux ou la peau rincez immédiatement avec de l’eau. Veuillez consulter tous conseils spécifiques dans les fiches de données de sécurité et/ou les règles de sécurité.
- Les réactifs sont réservés exclusivement au diagnostic et non pas au thérapeutique.
- Evitez de mélanger et d’échanger les réactifs de lots différents et de provenance différents.
- Evitez l’utilisation des réactifs perimés (voyez étiquette).
- N’exposez pas les réactifs à la lumière pendant le stockage ou l’incubation.
- Ne pas pipeter avec la bouche
- Ne pas manger, boire ou fumer dans les zones de manipulation de réactifs et d’échantillons.
- Evitez le contact de la peau et des muqueuses avec les réactifs.
- Pendant le travail avec les réactifs, utilisez des gants appropriés.
- Evitez le contact de substrats avec des métaux/oxydants.
- Evitez de gicler des liquides et la formation d’Aérosoles.
- A fin d’éviter des contaminations avec microbes ou contaminations de réactifs et d’échantillons qui pourraient rendre le test sans valeur, veuillez utiliser des pointes de pipettes jetables.
- Utilisez des tubes appropriés pour dispenser le conjugué et le substrat.
- Toute exposition aux acides inactive le conjugué.
- Pour la préparation des réactifs de l’eau déstillé ou déionisé doit être utilisée.
- La solution de substrat doit être rendue à température ambiante avant usage.
- Décontaminez et éliminez les échantillons et tous matériaux contaminés de manière comme si ils contenaient des germes de maladies infectieuses. La méthode préférée de décontamination est par l’autoclave pour au moins une heure à 121.5 °C.
- Traitez les déchets liquides non-acides tel que des déchets neutralisés par l’hypochlorite de sodium (concentration finale d’hypochlorite: 1,0%). Après 30 minutes le décontamination effective est atteinte. Les déchets liquides contenant de l’acide doivent être neutralisés avant la décontamination.
4. Préparation des Réactifs

Placer les concentrés de tampon à une température ambienne et diluer avant de commencer le test. Si des cristaux se sont formés dans les concentrés de tampon, chauffer doucement ces derniers jusqu'à fin de les dissoudre la dissolution des cristaux totale.

4.1. Tampon de Lavage (1x)

Verser tout le contenu (50 ml) du concentré de tampon de lavage (20x) dans un cylindre gradué propre de 1000 ml. Porter le volume final à 1000 ml avec de l'eau distillée ou déionisée dans un alambic en verre. Mélanger doucement pour éviter la formation de mousse.

Transférer tout dans une bouteille de lavage et conserver à une température comprise entre 2º et 25ºC. Noter que le tampon de lavage reste stable pendant 30 jours.

<table>
<thead>
<tr>
<th>Nombre de bandes</th>
<th>Tampon de lavage (20x) (ml)</th>
<th>Eau distillée (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>25</td>
<td>475</td>
</tr>
<tr>
<td>1 - 12</td>
<td>50</td>
<td>950</td>
</tr>
</tbody>
</table>

4.2. Tampon d’essai (1x)

Bien mélanger le contenu de la bouteille. Ajouter le contenu du tampon d’essai concentré (20x) (5.0 ml) aux 95 ml d'eau distillée ou déionisée et mélanger doucement pour éviter la formation de mousse. Stocker le tout entre 2º et 8ºC. Noter que le tampon d’essai reste stable pendant 30 jours.

La couleur rouge n'altère en aucun cas les résultats de tests. Elle a été conçue pour aider le client à réaliser le test.

Le tampon d’essai peut être préparé selon le tableau suivant :

<table>
<thead>
<tr>
<th>Nombre de bandes</th>
<th>Tampon d’essai (20x) (ml)</th>
<th>Eau distillée (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>2.5</td>
<td>47.5</td>
</tr>
<tr>
<td>1 - 12</td>
<td>5.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

4.3. Préparation du conjugué biotine

Noter que le conjugué biotine doit être utilisé dans les 30 minutes qui suivent la dilution.

Le conjugué biotine doit être dilué au 1:100 avec le tampon d’essai (1x) juste avant l'utilisation dans un tube à essais en plastique propre.

Le conjugué biotine peut être préparé selon le tableau suivant :

<table>
<thead>
<tr>
<th>Nombre de bandes</th>
<th>Conjugué biotine (ml)</th>
<th>tampon d’essai (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>2.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>
4.4. Streptavidine-HRP

Noter que la Streptavidine-HRP doit être utilisée dans les 30 minutes qui suivent la dilution.

La Streptavidine-HRP doit être diluée au 1:200 avec le tampon d’essai (1x) juste avant utilisation dans un tube à essais en plastique propre et mélanger doucement.

Le Streptavidine-HRP peut être préparé selon le tableau suivant :

<table>
<thead>
<tr>
<th>Nombre de bandes</th>
<th>Streptavidine-HRP (ml)</th>
<th>tampon d’essai (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>5.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>11.94</td>
</tr>
</tbody>
</table>

4.5. Étalon human IL-1 alpha

Reconstituer étalon human IL-1 alpha en ajoutant de l’eau distillée. Le volume de reconstitution est indiqué sur l’étiquette de flacon d’étalon.
Agiter et mélanger avec précaution pour assurer une solubilisation homogène complète (concentration d’étalon reconstitué = 200 pg/ml). Laisser reconstituer l’étalon pendant 10-30 min..

Après utilisation le surplus d’étalon ne doit pas être gardé et doit être éliminé.

Des dilutions d’étalon peuvent être préparées directement sur la plaque de microtitration (voir 5.c) ou comme alternative dans des tubes (voir 4.5.1).

4.5.1 Dilution d’étalon externe

Étiqueter les tubes 7, une pour chaque point d’étalon. S1, S2, S3, S4, S5, S6, S7
Puis préparer séries de dilutions 1:2 pour la courbe d’étalonnage de manière suivante: Pipeter 225 µl de diluant d’échantillon dans chaque tube.
Pipeter 225 µl d’étalon reconstitué (concentration d’étalon = 200 pg/ml) dans un premier tube marqué S1 et agiter (concentration d’étalon 1 = 100 pg/ml).
Pipeter 225 µl de cette dilution dans un deuxième tube marqué S2, et mélanger soigneusement avant le transfert suivant. Répéter des séries de dilutions 5 fois pour créer les dilutions d’étalon pour la courbe d’étalonnage (voir Figure 13).

Diluant d’échantillon sert comme contrôle vide.

Figure 13
5. Protocole de Test

1. Déterminer le nombre de barrettes de puits de microtitration nécessaires pour tester le nombre souhaité d’échantillons plus les barrettes nécessaires aux contrôles vides et aux étalons. Chaque échantillon, étalon, contrôle vide doit être testé en double. Retirer les barrettes de microtitration inutiles du support et les stocker à 2°-8°C dans une pochette hermétiquement refermée, avec le dessiccatif fourni.

2. Laver deux fois les barrettes de puits avec environ 400 µl de tampon de lavage pour chaque puits et en aspirant à fond le contenu des puits entre les lavages. Laisser le Tampon de lavage dans les puits pendant 10 - 15 secondes avant l’aspiration. Veiller à ne pas rayer la surface des puits de microtitration. Après le dernier lavage, vider les barrettes de puits et les tapoter sur un tampon absorbant ou une serviette en papier pour éliminer l’excès de tampon de lavage. Utiliser les barrettes de micropuits immédiatement après le lavage ou les placer renversées sur un papier absorbant pendant 15 minutes au maximum. Ne pas laisser sécher les puits.

3. Dilution d’étalon sur la plaque de microtitration (Comme alternative des dilutions d’étalon peuvent être préparées dans des tubes -voir 4.5.1) 
Ajouter en double 100 µl de diluant d’échantillon dans tous les puits d’étalon. Pipeter en double 100 µl d’étalon préparé (voir Préparation d’étalon 4.5, concentration = 200.0 pg /ml) dans les puits A1 et A2 (voir Table 6). Mélanger bien le contenu des puits A1 et A2 par aspiration et éjection répétée (concentration d’étalon 1, S1 = 100.0 pg/ml), et transférer 100 µl dans les puits B1 et B2, respectivement (voir Figure 14). Veiller à ne pas rayer la surface des puits de microtitration. Continuer la procédure 5 fois en préparant deux séries de dilutions d’étalon human IL-1 alpha, de 100.0 à 1.6 pg/ml. Eliminer 100 µl du contenu des derniers puits (G1, G2).

Figure 14

Dans le cas d’une dilution d’étalon externe (voir 4.5.1), pipeter 100 µl de ces dilutions d’étalon (S1 – S7) dans les puits de façon montrée dans Table 6.

4. Ajouter 100 µl de diluant d’échantillon dans tous les puits de contrôle vide.
Table 6
Exemple d’arrangement d’échantillons, d’étalons et de contrôles vides dans les barrettes de puits de microtitration.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Étalon 1 (100.0 pg/ml)</td>
<td>Étalon 1 (100.0 pg/ml)</td>
<td>Échantillon 1</td>
<td>Échantillon 1</td>
</tr>
<tr>
<td>B</td>
<td>Étalon 2 (50.0 pg/ml)</td>
<td>Étalon 2 (50.0 pg/ml)</td>
<td>Échantillon 2</td>
<td>Échantillon 2</td>
</tr>
<tr>
<td>C</td>
<td>Étalon 3 (25.0 pg/ml)</td>
<td>Étalon 3 (25.0 pg/ml)</td>
<td>Échantillon 3</td>
<td>Échantillon 3</td>
</tr>
<tr>
<td>D</td>
<td>Étalon 4 (12.5 pg/ml)</td>
<td>Étalon 4 (12.5 pg/ml)</td>
<td>Échantillon 4</td>
<td>Échantillon 4</td>
</tr>
<tr>
<td>E</td>
<td>Étalon 5 (6.3 pg/ml)</td>
<td>Étalon 5 (6.3 pg/ml)</td>
<td>Échantillon 5</td>
<td>Échantillon 5</td>
</tr>
<tr>
<td>F</td>
<td>Étalon 6 (3.1 pg/ml)</td>
<td>Étalon 6 (3.1 pg/ml)</td>
<td>Échantillon 6</td>
<td>Échantillon 6</td>
</tr>
<tr>
<td>G</td>
<td>Étalon 7 (1.6 pg/ml)</td>
<td>Étalon 7 (1.6 pg/ml)</td>
<td>Échantillon 7</td>
<td>Échantillon 7</td>
</tr>
<tr>
<td>H</td>
<td>Contrôle vide</td>
<td>Contrôle vide</td>
<td>Échantillon 8</td>
<td>Échantillon 8</td>
</tr>
</tbody>
</table>

5. Ajouter 100 µl de diluant d’échantillon dans tous les puits d’échantillon.
6. Ajouter 50 µl de chaque échantillon, en double, dans les puits d’échantillon.
7. Préparer du Conjugué Biotine (se reporter à la préparation des réactifs Conjugué Biotine 4.3).
8. Ajouter 50 µl de Conjugué Biotine dans tous les puits.
9. Recouvrir avec un couvre-plaque et incuber à température ambiante (entre 18° et 25°C) pendant 2 heures, si possible sur un agitateur rotateur réglé à 200 tr/min.
10. Préparer Streptavidine-HRP (se reporter à la préparation des réactifs Streptavidine-HRP 4.4)
11. Retirer le couvre-plaque et vider les puits. Laver 4 fois les barrettes de puits de microtitration comme indiqué à point b de ce protocol. Utiliser les barrettes de micropuits immédiatement après le lavage.
12. Ajouter 100 µl de Streptavidine-HRP dans tous les puits.
13. Recouvrir avec un couvre-plaque et incuber à température ambiante (entre 18 et 25°C) pendant 1 heure, si possible sur un agitateur rotateur réglé à 200 tr/min.
14. Retirer le couvre-plaque et vider les puits. Laver 4 fois les barrettes de puits de microtitration comme indiqué à point b de ce protocol. Utiliser les barrettes de micropuits immédiatement après le lavage.
15. Pipeter 100 µl de solution de substrat TMB dans chaque puits, y compris les puits de contrôle vide.
16. Incuber les puits de microtitration à température ambiante (entre 18 et 25°C) pendant environ 10 minutes. Éviter toute exposition directe à une source de lumière intense.

Les valeurs de densité optique au niveau de la plaque doivent être surveillées et la réaction du substrat stoppée (voir le point prochain) avant que les puits positifs ne soient plus correctement mesurables.
La durée de l’incubation pour le développement de couleur doit être déterminé pour chaque essai individuellement.
Il est recommandé d’ajouter la solution stop quand une couleur bleu sombre se développe à la concentration la plus haute de la gamme étalon. Une autre alternative consiste à suivre le développement de la couleur par lecteur ELISA à 620 nm. La réaction du substrat doit être arrêtée dès que la DO atteint 0.9 à 0.95.

17. Arrêter la réaction enzymatique en pipetant rapidement 100 µl de solution d'arrêt dans chaque puits, y compris les puits de contrôle vide. Il est important que la solution d'arrêt soit répandue rapidement et uniformément dans les puits pour inactiver complètement l'enzyme. Les résultats doivent être lus immédiatement après l'ajout de la solution d'arrêt ou dans l'heure qui suit si les barrettes de microtitration sont conservées à l'obscurité entre 2 et 8 °C.

18. Lire l'absorbance de chaque puits sur un spectrophotomètre avec 450 nm comme longueur d'onde primaire (éventuellement 620 nm comme longueur d'onde de référence; 610 à 650 nm sont acceptables). Mesurer le contrôle vide du lecteur de plaque conformément aux instructions du fabricant, en utilisant les puits de contrôle vide. Déterminer l'absorbance des échantillons et des human IL-1 alpha.

Les échantillons ont été diluées 1:2 en cours de test. Pour cette raison, la valeur de concentration déterminée par la gamme étalon doit être multipliée par le facteur de dilution (x 2).

**Note:** Si la plaque n’est pas agitée pendant l’incubation, les valeurs de densité optique peuvent être inférieur aux valeurs indiqués plus haut. Néanmoins ces valeurs sont valables.
INFORMAZIONI SUL PRODOTTO E MANUALE (italiano)

1. Reagenti Forniti

1.1. Reagenti Forniti per human IL-1 alphaELISA BMS243/2CE (96 tests)

1. busta d’alluminio con una **Piastra Micropozzetti rivestita** con anticorpo policlonale anti human IL-1 alpha
2. flaconcino (70 µl) di anticorpo **Biotina Coniugato** (anticorpo policlonale human IL-1 alpha)
3. flaconcino (150 µl) di **Streptavidina-HRP**
4. flaconcini human IL-1 alpha **Standard** liofilizzato, 200 pg/ml previa ricostituzione diluizione
5. flaconcino (12 ml) con **Diluente del Campione**

**Attenzione:** In alcuni rari casi un precipitato insolubile di stabilizzatore della proteina potrebbe essere visibile nella fiala. Questo precipitato non interferisce in alcun modo con il buon risultato dei test e può quindi essere ignorato.

6. flaconcino (5 ml) con **Tampone del Saggio concentrata** 20x (PBS con 1% Tween 20 e 10% BSA)
7. bottiglia (50 ml) con **Tampone di Lavaggio concentrato** 20x (PBS con 1% Tween 20)
8. flaconcino (15 ml) di **Soluzione Substrato** (tetrametilbenzidina)
9. flaconcino (15 ml) di **Soluzione bloccante** (acido fosforico 1M)
10. **Copripiastra** adesivi

1.2. Reagenti Forniti per human IL-1 alphaELISA BMS243/2TENCE (10x96 tests)

1. busta d’alluminio con una **Piastra Micropozzetti rivestita** con anticorpo policlonale anti human IL-1 alpha
2. flaconcini (70 µl) di anticorpo **Biotina Coniugato** (anticorpo policlonale verso human IL-1 alpha)
3. flaconcini (150 µl) di **Streptavidina-HRP**
4. flaconcini human IL-1 alpha **Standard** liofilizzato, 200 pg/ml previa ricostituzione diluizione
5. flaconcini (12 ml) con **Diluente del Campione**

**Attenzione:** In alcuni rari casi un precipitato insolubile di stabilizzatore della proteina potrebbe essere visibile nella fiala. Questo precipitato non interferisce in alcun modo con il buon risultato dei test e può quindi essere ignorato.

6. flaconcini (5 ml) con **Tampone del Saggio concentrata** 20x (PBS con 1% Tween 20 e 10% BSA)
7. bottiglie (50 ml) con **Tampone di Lavaggio concentrato** 20x (PBS con 1% Tween 20)
8. flaconcini (15 ml) di **Soluzione Substrato** (tetrametilbenzidina)
9. flaconcino (100 ml) di **Soluzione bloccante** (acido fosforico 1M)
10. **Copripiastra** adesivi
2. Istruzioni di Conservazione

Conservare i reagenti del kit a 2°-8° C. Subito dopo l'uso riporre i reagenti nel luogo di conservazione a 2°-8° C. La scadenza del kit e dei reagenti è indicata sulle etichette.
La data di scadenza dei componenti del kit può essere garantita solo se questi sono conservati correttamente e, in caso di uso ripetuto di un componente, il reagente non è stato contaminato durante la prima manipolazione.

3. Precauzioni per l'Uso

- I reagenti sono per uso in vitro diagnostico e non sono per uso terapeutico.
- Non mischiare tra loro reagenti di diversi lotti o provenienza.
- Non usare i kit dopo la data di scadenza.
- Non esporre i reagenti del kit, durante la conservazione e incubazione a forti fonti di luce.
- Non pipettare utilizzando la bocca.
- Non mangiare o fumare nell'area dove sono utilizzati i reagenti dei kit o i campioni.
- Evitare il contatto dei reagenti o campioni con la pelle o le mucose.
- Guanti di gomma o lattice dovrebbero essere sempre indossati quando si usano reagenti e campioni.
- Evitare il contatto tra il substrato del kit e agenti ossidanti e metallo.
- Evitare schizzi o produzione di aereosol.
- Per evitare contaminazione microbiaca o cross-contaminazione dei reagenti o dei campioni che invaliderebbero il test, usare sempre pipette e puntali mono-uso.
- Usare vaschette pulite e dedicate per la dispensare il reagente substrato.
- L'esposizione agli acidi inattiva il coniugato.
- Acqua distillata o de-ionizzata deve essere utilizzata per la preparazione dei reagenti.
- La soluzione di substrato deve essere portata a temperatura ambiente prima dell'utilizzo.
- Decontaminare ed eliminare i campioni e tutto il materiale potenzialmente contaminante perchè potrebbero contenere agenti infettanti. Il metodo preferito per la decontaminazione è l'autoclavaggio per minimo 1 ora a 121,5°C.
- Gli scarti liquidi, non contenenti acido e gli scarti neutralizzati possono essere mischiati con sodio ipoclorido in un volume finale di 1,0%. Lasciare minimo 30 minuti per l'effettiva decontaminazione. Scarti liquidi contenenti acido devono essere neutralizzati prima dell'aggiunta di sodio ipoclorido.
4. Preparazione dei Reagenti

Prima di cominciare con le procedure del test i concentrati dei tamponi devono essere portati a temperatura ambientale e diluiti alle concentrazioni adeguate. Se i concentrati dei tampone presentano cristalli in sospensione, riscaldare lievemente i tamponi fino a ottenere la completa dissoluzione dei cristalli.

4.1. Tampone di Lavaggio (1x)

Versare l'intero contenuto (50 ml) del tampone di lavaggio concentrato (20x) in un cilindro graduato pulito da 1000 ml. Portare il volume finale a 1000 ml utilizzando acqua distillata o acqua deionizzata. Mescolare delicatamente per evitare la formazione di schiuma.

Trasferire il prodotto in una bottiglia pulita e conservare a temperature comprese fra 2°C e 25°C. Il tampone di lavaggio è stabile per 30 giorni.

Se necessario, è possibile preparare il tampone di lavaggio secondo la tabella seguente:

<table>
<thead>
<tr>
<th>Numero di strip</th>
<th>Tampone di lavaggio (20x) (ml)</th>
<th>Acqua distillata (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>25</td>
<td>475</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Numero di strip</th>
<th>Tampone di lavaggio (20x) (ml)</th>
<th>Acqua distillata (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 12</td>
<td>50</td>
<td>950</td>
</tr>
</tbody>
</table>

4.2. Tampone del Saggio (1x)

Versare l'intero contenuto (5 ml) del tampone del saggio concentrato (20x) in un cilindro graduato pulito da 100 ml. Portare il volume finale a 100 ml utilizzando acqua distillata o acqua deionizzata. Mescolare delicatamente per evitare la formazione di schiuma.

Conservare a temperatura compresa fra 2°C e 8°C. La soluzione tampone diluita è stabile per 30 giorni.

Se necessario, è possibile preparare la soluzione tampone secondo la tabella seguente:

<table>
<thead>
<tr>
<th>Numero di strip</th>
<th>Tampone del Saggio (20x) (ml)</th>
<th>Acqua distillata (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>2.5</td>
<td>47.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Numero di strip</th>
<th>Tampone del Saggio (20x) (ml)</th>
<th>Acqua distillata (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 12</td>
<td>5.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

4.3. Biotina Coniugato

Il biotina coniugato deve essere utilizzato entro 30 minuti dalla diluizione.

Il biotina coniugato deve essere diluito 1:100 con Tampone del Saggio (1x) in una provetta di plastica pulita secondo la tabella seguente:

<table>
<thead>
<tr>
<th>Numero di strip</th>
<th>Biotina coniugato (ml)</th>
<th>Tampone del Saggio (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>2.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>
4.4. Streptavidina-HRP

Il Streptavidina-HRP deve essere utilizzato entro 30 minuti dalla diluizione.
Il Streptavidina-HRP deve essere diluito 1:200 con Tampone del Saggio (1x) in una provetta di plastica pulita secondo la tabella seguente:

<table>
<thead>
<tr>
<th>Numero di strip</th>
<th>Streptavidina-HRP (ml)</th>
<th>Tampone del Saggio (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>5.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>11.94</td>
</tr>
</tbody>
</table>

4.5. Human IL-1 alpha Standard

Ricostituire lo human IL-1 alpha standard aggiungendo il acqua distillata.

Dopo l'uso, lo standard rimanente non può essere riutilizzato e deve essere buttato.

La diluizione dello standard può essere fatto direttamente nella piastra (vedi 5.c.) oppure nei tubi (vedi 4.5.1).

4.5.1. Diluizione degli Standard esterni

Etichettare 7 tubi, uno per ogni punto dello standard.
S1, S2, S3, S4, S5, S6, S7

Preparare diluizione seriali 1:2 per lo standard nel seguente modo: Pipettare 225 ul di diluente del campione nei tutti tubi.
Pipettare 225 ul di standard ricostituito (concentrazione dello standard = 200 pg/ml) nel primo tubo, etichettato S1, e mescolare (concentrazione dello standard 1 = 100 pg/ml).
Pipettare 225 ul di questa diluizione nel secondo tubo, etichettato S2, mischiare accuratamente prima del successivo trasferimento. Ripetere le 5 diluizioni seriali in modo da creare i punti della curva di calibrazione (vedere Figure 15)

Diluente del campione serve come bianco.

Figure 15

![Diagram](image)

5. Procedura del Test

1. Stabilire il numero di strip dei micropozzetti necessarie per analizzare la quantità desiderata di campioni più le
strip per i bianchi e gli standard. Tutti i campioni, gli standardi, il bianco e il devono essere processati in duplicato. Rimuovere dal supporto le strip micropozzetti non utilizzate e conservarle nella bustina metallica contenente la polvere essiccante, mantenendole a 2°-8°C e perfettamente sigillate.


Dopo l'ultimo lavaggio, asciugare le strip micropozzetti con un tampone o carta assorbente e conservarle nella bustina metallica contenente la polvere essiccante, mantenendole a 2°-8°C e perfettamente sigillate.

Non lasciar asciugare i pozzetti.

3. **Diluizione dello standard in micropozzetti** (alternativamente la diluizione dello standard può avvenire in tubi – vedi 4.5.1)

Aggiungere 100 ul di diluente del campione in duplicato a tutti i pozzetti dello standard. Pipettare 100 ul standard preparato (vedi preparazione dello standard 4.5., concentrazione = 200.0 pg/ml) in duplicato nei pozzetti A1 e A2 (vedi tabella 1). Mescolare il contenuto dei pozzetti A1 e A2 attraverso ripetute aspirazione ed iniezioni (concentrazione dello standard 1, S1 = 100.0 pg/ml) e trasferire 100 ul, rispettivamente, ai pozzetti B1 e B2 (vedere Figure 16). Fare attenzione a non graffiare la parte interna dei pozzetti. Continuare questa procedura per 5 volte, creando due colonne di standard in diluizione con concentrazione da 100.0 a 1.6 pg/ml. Buttare 100 µl del contenuto degli ultimi pozzetti (G1 e G2).

In caso di **diluizione esterna dello standard** (vedi 4.5.1) pipettare 100 ul di queste diluizioni standard (S1 – S7) nei pozzetti degli standard come da tabella 1.

**Figure 16**

![Diagram](attachment:image.png)

4. Dispensare 100 µl di **diluente del campione** in duplicato ai **pozzetti de bianco**.

5. Dispensare 50 µl di **diluente del campione** in duplicato ai **pozzetti dei campioni**.

6. Dispensare 50 µl di **campione** in duplicato ai **pozzetti dei campioni**.
Table 7
Tabella rappresenta un esempio dell'organizzazione dei bianchi, standardi e campioni nei pozzetti:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard 1 (100.0 pg/ml)</td>
<td>Standard 1 (100.0 pg/ml)</td>
<td>Campione 1</td>
<td>Campione 1</td>
</tr>
<tr>
<td>B</td>
<td>Standard 2 (50.0 pg/ml)</td>
<td>Standard 2 (50.0 pg/ml)</td>
<td>Campione 2</td>
<td>Campione 2</td>
</tr>
<tr>
<td>C</td>
<td>Standard 3 (25.0 pg/ml)</td>
<td>Standard 3 (25.0 pg/ml)</td>
<td>Campione 3</td>
<td>Campione 3</td>
</tr>
<tr>
<td>D</td>
<td>Standard 4 (12.5 pg/ml)</td>
<td>Standard 4 (12.5 pg/ml)</td>
<td>Campione 4</td>
<td>Campione 4</td>
</tr>
<tr>
<td>E</td>
<td>Standard 5 (6.3 pg/ml)</td>
<td>Standard 5 (6.3 pg/ml)</td>
<td>Campione 5</td>
<td>Campione 5</td>
</tr>
<tr>
<td>F</td>
<td>Standard 6 (3.1 pg/ml)</td>
<td>Standard 6 (3.1 pg/ml)</td>
<td>Campione 6</td>
<td>Campione 6</td>
</tr>
<tr>
<td>G</td>
<td>Standard 7 (1.6 pg/ml)</td>
<td>Standard 7 (1.6 pg/ml)</td>
<td>Campione 7</td>
<td>Campione 7</td>
</tr>
<tr>
<td>H</td>
<td>Bianco</td>
<td>Bianco</td>
<td>Campione 8</td>
<td>Campione 8</td>
</tr>
</tbody>
</table>

7. Preparare la biotina coniugato (consultare la sezione biotina coniugato 4.3 sulla preparazione dei reagenti).

8. Dispensare 50 µL di biotina coniugato a ciascun pozzetto.

9. Coprire con un copripiastra e incubare a temperatura ambiente (18°-25°C) per 2 ore utilizzando, se disponibile, un vortex a 400 rpm.


12. Dispensare 100 µL di streptavidina-HRP a ciascun pozzetto.

13. Coprire con un copripiastra e incubare a temperatura ambiente (18°-25°C) per 1 ora utilizzando, se disponibile, un vortex a 400 rpm.


15. Pipettare 100 µL di soluzione substrato TMB in tutti i pozzetti, inclusi quelli del blank.

16. Incubare le strip a temperatura ambiente (18°-25°C) per circa 10 minuti. Evitare l'esposizione diretta a luci intense.

È necessario monitorare i valori O.D. a livello della piastra e interrompere la reazione del substrato (vedi il punto prossimo del protocollo) prima che i pozzetti positivi cessino di essere appropriatamente registrabili. La determinazione del tempo necessario per lo sviluppo del colore dev'essere fatto per ogni singolo parametro.

Si raccomanda di aggiungere la soluzione di stop quando lo standard più elevato ha sviluppato un colore blu scuro.

Alternativamente lo sviluppo del colore può essere monitorato con un lettore ELISA a 620 nm. La reazione del substrato deve essere bloccata non appena viene misurato un valore delle OD di 0.9 - 0.95.
17. Interrompere la reazione enzimatica pipettando rapidamente 100 µl di soluzione bloccante in ciascun pozzetto, inclusi i pozzetti del bianco. È importante che la soluzione bloccante si diffonda rapidamente e uniformemente attraverso i micropozzetti per inattivare completamente l’enzima. I risultati devono essere letti immediatamente dopo l’aggiunta della soluzione bloccante o entro 1 ora se le strip sono conservate in un luogo buio a 2°-8° C.

18. Leggere l’assorbanza di ciascun micropozzetto su uno spettrofotometro che utilizza 450 nm come lunghezza d’onda primaria (620 nm come lunghezza d’onda di riferimento alternativa; valori da 610 nm a 650 nm sono accettabili). Azzerare il lettore della piastra secondo le istruzioni del produttore e utilizzando i pozzetti del bianco. Determinare l’assorbanza sia dei campioni, sia degli standard di human IL-1 alpha.

I campioni sono stati diluiti 1: 2, quindi la concentrazione dalla curva standard risultante deve essere moltiplicata per il fattore di diluizione (x 2).

Note: In caso di incubazione senza agitazione i valori di densità ottica (O.D.) potranno essere più bassi di quanto indicato sotto. Tuttavia i risultati saranno da ritenersi validi.
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