Immunoassay Kit
Catalog #KPC0081

Monkey
IL-8

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1. INTENDED USE

The monkey IL-8 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of monkey Interleukin-8 levels in cell culture supernatants and monkey serum. **The monkey IL-8 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2. SUMMARY

Interleukin-8/Neutrophil-Activating Peptide-1 selectively stimulates the ability of neutrophils and T-lymphocytes to invade injured or inflamed tissue (1,9,11,13). Exogenous stimuli like LPS (10), but also IL-1, TNF-α and TNF-β induce the secretion of IL-8 (8) in a variety of different cell types including monocytes, endothelial and epithelial cells, peripheral blood mononuclear cells, dermal fibroblasts (8), keratinocytes (8), neutrophils (3), hepatocytes, synovial cells (4), and T-lymphocytes (11). When IL-8 was subcutaneously injected into rats, both lymphocytes and neutrophils migrated to the site of injection within 3 hours. At lower dosages, only lymphocytes migrated towards the site of injection, while at higher dosages primarily neutrophils were attracted. It was found that T-lymphocytes are 10 times more sensitive to IL-8 than neutrophils. IL-8 exerts its effects via specific cell membrane receptors (chemotactic agonist receptor-family) with homogeneous high-affinity activity and two binding sites for its ligand (2,7). The receptor density is determined by the cell type and ranges from 300 on T-lymphocytes up to 20,000 on neutrophils (11). After binding of IL-8, the receptor expression is downregulated >90% within 10 minutes at 37°C, together with the internalization of the ligand (11,12). IL-8 is proteolytically degraded in the cytoplasm and released into the culture medium as soluble fragments (11). The IL-8 receptors

are probably recycled (12). Besides its chemotactic influence, IL-8 exerts other distinct characteristics. In neutrophils it triggers the secretion of superoxide anions and lysosomal enzymes, thereby indirectly augmenting the permeability of blood vessels (14), and IL-8 enhances the fungicidal activity against *Candida albicans.* Neutrophils are more readily liberated from the bone marrow reservoir under the influence of this cytokine (11). *In vitro*, IL-8 stimulates a rapid Mac-1 as well as CR 1, p150,95 and LFA-1 expression on neutrophils which enables the adherence to activated vascular endothelial cells expressing e.g. ICAM-1 (5). This may account for the accumulation of neutrophils at IL-8 injection sites. Other findings suggest that endothelial-derived IL-8 may function to attenuate inflammatory events at the interface between vessel wall and blood, via inhibiting neutrophil adhesion to cytokine-activated endothelial monolayers. Therefore these cells seem to be protected against neutrophil-mediated damage (6). In basophils, besides its chemotactic effects, IL-8 stimulates the histamine liberation.

The property of IL-8 to stimulate movement of neutrophils across endothelial monolayers *in vitro* supports the concept of a central role for this molecule in the accumulation of neutrophils at inflammatory lesions *in vivo.*

3. PRINCIPLE OF THE TEST

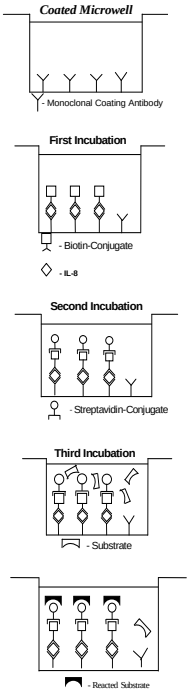
An anti-monkey-IL-8 monoclonal coating antibody is adsorbed onto microwells.

Monkey IL–8 present in the sample or standard binds to antibodies adsorbed to the microwells; a Biotin-conjugated anti-monkey-IL-8 antibody is added and binds to monkey IL-8 captured by the first antibody.

Following incubation, unbound biotin conjugated anti-monkey-IL-8 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-monkey-IL-8.

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

A colored product is formed in proportion to the amount of monkey IL-8 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven monkey IL-8 standard dilutions and monkey IL-8 sample concentration determined.



4. REAGENTS PROVIDED

Reagent	*96 Test Kit*
Aluminium pouch with microwell plate coated with monoclonal antibody (murine) to monkey IL-8.	1 plate
Biotin-Conjugate anti-monkey-IL-8 polyclonal antibody; 0.1 mL per vial.*	1 vial
Streptavidin-HRP Concentrate; 150 µL per vial.*	1 vial
Monkey IL-8 Standard Concentrate, 125,000 U/mL; 100 µL per vial.*	2 vials
Wash Buffer Concentrate 20x (PBS with 1% Tween 20); 50 mL per bottle.*	1 bottle
Assay Buffer Concentrate 20x (PBS with 1% Tween 20 and 10% BSA); 5 mL per vial.*	1 vial
Sample Diluent; 12 mL per bottle.*	1 bottle
Substrate Solution (tetramethylbenzidine); 15 mL per vial.	1 vial
Stop Solution (1 M phosphoric acid); 12 mL per vial.	1 vial
Blue-Dye, Green-Dye, Red-Dye; 0.4 mL per vial.*	3 vials
Adhesive Plate Covers.	4
* reagents contain preservative (0.01% Proclin® 300).	

5. STORAGE INSTRUCTIONS

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

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

6. SPECIMEN COLLECTION

Cell culture supernatants and monkey serum (cynomolgus, baboon) are suitable for use in the assay. Remove the serum from the clot as soon as possible after clotting and separation.

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

Samples must be stored at 2 to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive monkey IL-8. Addition of protease inhibitors may provide better stability of samples. Avoid repeated freeze-thaw cycles.

For sample stability refer to 13.5.

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 multi-channel micropipette with disposable tips.
- Multi-channel micropipette reservoir.
- Beakers, flasks, cylinders necessary for preparation of reagents.
- Device for delivery of wash solution (multi-channel micropipette, wash bottle or automatic wash system).
- Microwell strip reader capable of measurement 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 chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techinques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory coat, 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 research use only and are not for use in diagnostic or 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.
- 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 reagents.
- Exposure to acids will inactivate 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 of specimens and all potentially contaminated materials as if 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.

9.1. Wash Buffer

If crystals have formed in the *Wash Buffer Concentrate*, warm it gently until they have completely dissolved.

Pour entire contents (50 mL) of the *Wash Buffer Concentrate* into a clean 1,000 mL graduated cylinder. Bring final volume to 1,000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. Adjust the final pH of the solution to 7.4.

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

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

9.2. Assay Buffer (1x)

Mix the contents of the bottle well. Add contents of *Assay Buffer Concentrate* (20x) (5 mL) to 95 mL distilled or deionized water and 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 may be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

9.3. Preparation of Biotin-Conjugate

The Biotin-Conjugate must be diluted 1:100 with Assay Buffer (1x) just prior to use in a clean plastic test tube.

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution. Biotin-Conjugate may be prepared as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

9.4. Preparation of Streptavidin-HRP

Make a 1:200 dilution of the concentrated Streptavidin-HRP solution as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (mL)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

Please note: The Streptavidin-HRP should be used within 30 minutes after dilution.

9.5. Preparation of Monkey IL-8 Standard

The concentrated monkey IL-8 Standard must be diluted 1:50 with Assay Buffer (1x) just prior to use in a clean plastic test tube according to the following dilution scheme:

> 20 µL concentrated monkey IL-8 Standard + 980 µL Assay Buffer (1x). Shake gently to mix (concentration of diluted standard = 2500 U/mL).

Please note: In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the standard and conjugate vials. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.

9.6. Addition of color-giving reagents: Blue-Dye, Green-Dye, Red-Dye

In order to help our customers to avoid any mistakes in pipetting, this kit offers a tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colors to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (*Blue-Dye*, *Green-Dye*) can be added to the reagents according to the following guidelines:

1. Diluent: Before sample dilution, add the *Blue-Dye* at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of *Blue-Dye*, proceed according to the instruction booklet.

5 mL Diluent	20 µL *Blue-Dye*
12 mL Diluent	48 µL *Blue-Dye*

2. Biotin-Conjugate: Before dilution of the concentrated conjugate, add the *Green-Dye* at a dilution of 1:100 (see table below) to the *Assay Buffer* used for the final conjugate dilution. Proceed after addition of *Green-Dye* according to the instruction booklet, preparation of Biotin-conjugate.

3 mL Assay Buffer	30 µL *Green-Dye*
6 mL Assay Buffer	60 µL *Green-Dye*
12 mL Assay Buffer	120 µL *Green-Dye*

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP; add the *Red-Dye* at a dilution of 1:250 (see table below) to the Assay Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of *Red-Dye* according to the instruction booklet, preparation of Streptavidin-HRP.

6 mL Assay Buffer	24 µL *Red-Dye*
12 mL Assay Buffer	48 µL *Red-Dye*

10. TEST PROTOCOL

a. Mix all reagents thoroughly without foaming before use.

b. 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 coated with Monoclonal Antibody* (murine) to monkey IL-8 from holder and store in foil bag with the desiccant provided at 2 to 8°C sealed tightly.

c. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

   After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µL of *Sample Diluent*, in duplicate, to all standard wells. Prepare standard dilutions by pipetting 100 µL of diluted (refer to preparation of reagents, 9.5.) *monkey IL-8 Standard*, in duplicate, into wells A1 and A2 (see Figures 1 and 2). Mix the contents by repeated aspiration and ejection and transfer 100 µL to wells B1 and B2, respectively. Continue this procedure five times, creating two rows of monkey-IL-8 standard dilutions ranging from 1250 to 19 U/mL. Discard 100 µL of the contents from the last microwells used (G1, G2).

Figure 1. Preparation of monkey IL-8 standard dilutions:

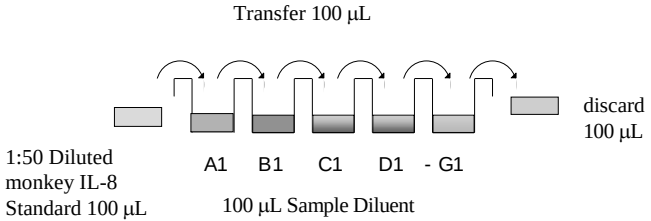


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (1250 U/mL)	Standard 1 (1250 U/mL)	Sample 1	Sample 1
B	Standard 2 (625 U/mL)	Standard 2 (625 U/mL)	Sample 2	Sample 2
C	Standard 3 (313 U/mL)	Standard 3 (313 U/mL)	Sample 3	Sample 3
D	Standard 4 (156 U/mL)	Standard 4 (156 U/mL)	Sample 4	Sample 4
E	Standard 5 (78 U/mL)	Standard 5 (78 U/mL)	Sample 5	Sample 5
F	Standard 6 (39 U/mL)	Standard 6 (39 U/mL)	Sample 6	Sample 6
G	Standard 7 (19 U/mL)	Standard 7 (19 U/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µL of *Sample Diluent*, in duplicate, to the blank wells.

f. Add 50 µL of *Sample Diluent* to all wells designated for samples.

g. Add 50 µL of each *Sample*, in duplicate, to the designated wells and mix the contents.

h. Prepare *Biotin-Conjugate* (refer to preparation of reagents, 9.3.).

i. Add 50 µL of diluted *Biotin-Conjugate* to all wells, including the blank wells.

j. Cover with a *Plate Cover* and incubate at room temperature (18 to 25°C) for 2 hours, on a rotator set at 100 rpm, if available.

k. Remove *Plate Cover* and empty wells. Wash microwell strips 3 times according to point c. of the protocol. Proceed immediately to the next step.

l. Prepare *Streptavidin-HRP* (refer to preparation of reagents, 9.4.).

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

n. Cover with a ***Plate Cover*** and incubate at room temperature (18 to 25°C) for 1 hour, on a rotator set at 100 rpm, if available.

o. Remove *Plate Cover* and empty wells. Wash microwell strips 3 times according to point c. of the protocol. Proceed immediately to the next step.

P. Pipette 100 µL of *TMB Substrate Solution* into all wells, including the blank wells.

q. Incubate the microwell strips at room temperature (18 to 25°C) for about 10 minutes, on a rotator set at 100 rpm, if available. Avoid direct exposure to intense light. **The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly recordable.** It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an O.D. of 0.6 - 0.65 is reached.

r. Stop the enzyme reaction by quickly pipetting 100 µL of *Stop Solution* into each well, including the blank wells. 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 to 8°C in the dark.

s. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 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 monkey IL-8 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 percent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the monkey IL-8 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

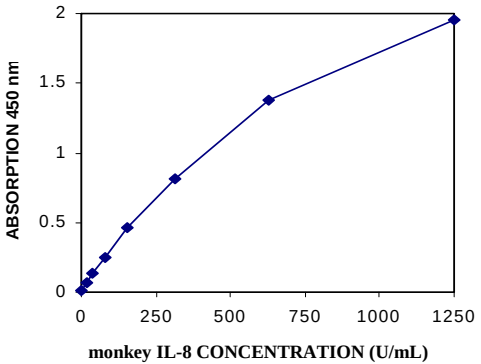
- To determine the concentration of circulating monkey IL-8 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding monkey IL-8 concentration.

- **For samples which have been diluted according to the instructions given in this manual, 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x2).**

  Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low monkey IL-8 levels. Such samples require further dilution of 1:10 (90 µL Assay Buffer + 10 µL Sample) or even higher in order to precisely quantitate the actual monkey IL-8 level.

- It is suggested that each testing facility establish a control sample of known monkey IL-8 concentration and run this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.
- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for monkey IL-8 ELISA. Monkey IL–8 was diluted in serial two-fold steps in Assay Buffer; symbols represent the mean of three parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the monkey IL-8 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Monkey IL-8 concentration (U/mL)	O.D. Mean	C.V. (%)
1	1250	1.967	1.8
	1250		
2	625	1.513	0.6
	625		
3	313	1.132	3.8
	313		
4	156	0.769	2.3
	156		
5	78	0.490	3.6
	78		
6	39	0.279	4.7
	39		
7	19	0.165	1.2
	19		
Blank	0	0.040	
	0		

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. Completely empty wells before dispensing fresh Wash Buffer, 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 monkey IL-8 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 3 U/mL (mean of 6 independent assays).

13.2. Reproducibility

13.2.1. Intra-assay

Reproducibility within the assay was evaluated in independent experiments. The overall intra-assay coefficient of variation has been calculated to be <6.3%.

13.2.2. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in independent experiments. The overall inter-assay coefficient of variation has been calculated to be <8.7%.

13.3. Spike Recovery

The spike recovery was evaluated by spiking four levels of monkey IL-8 into pooled monkey serum. Recoveries ranged from 72-125% with an overall mean recovery of 88%.

13.4. Dilution Parallelism

Four serum samples with different levels of monkey IL-8 were analyzed at serial two-fold dilutions (1:2 to 1:16) with 4 replicates each. Recoveries ranged from 90-119% with an overall mean recovery of 107%.

13.5. Sample Stability

13.5.1. Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed up to 5 times, and monkey IL-8 levels determined. There was no significant loss of monkey IL-8 by freezing and thawing.

13.5.2. Storage Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C, 2 to 8°C, room temperature (RT) and at 37°C, and the monkey IL-8 levels determined after 24 hours. There was no loss of monkey IL-8 immunoreactivity during storage under these conditions.

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15. ORDERING INFORMATION

Please address your orders to:

www.invitrogen.com
Invitrogen Corporation
Carlsbad, California 92008
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

16. REAGENT PREPARATION SUMMARY

A. Wash Buffer

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

B. Assay Buffer

Number of Strips	Assay Buffer Concentr. (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Biotin-Conjugate

Make a 1:100 dilution according to the table.

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Standard

Dilute concentrated standard 1:50 by mixing 20 µL concentrated monkey IL-8 Standard + 980 µL Assay Buffer.

E. Streptavidin-HRP

Make a 1:200 dilution according to the table.

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (mL)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

17. TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer.**
- Add 100 µL **Sample Diluent**, in duplicate, to standard wells.
- Pipette 100 µL diluted **monkey IL-8 Standard** into the first wells and create standard dilutions ranging from 1250 to 19 U/mL by transferring 100 µL from well to well. Discard 100 µL from the last well.
- Add 100 µL **Sample Diluent**, in duplicate, to the blank wells.
- Add 50 µL **Sample Diluent** to sample wells.
- Add 50 µL **Sample**, in duplicate, to designated wells.
- Prepare **Biotin-Conjugate**.
- Add 50 µL of diluted **Biotin-Conjugate** to all wells.
- Cover microwell strips and incubate 2 hours at room temperature (18 to 25°C).
- Prepare **Streptavidin-HRP**.
- Empty and wash microwell strips 3 times with **Wash Buffer.**
- Add 100 µL of diluted **Streptavidin-HRP** to all wells.
- Cover microwell strips and incubate 1 hour at room temperature (18 to 25°C).
- Empty and wash microwell strips 3 times with **Wash Buffer.**
- Add 100 µL of **TMB Substrate Solution** to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18 to 25°C).

- Add 100 µL **Stop Solution** to all wells including blank wells.
- Blank microwell reader and measure color intensity at 450 nm.

Note: For samples which have been diluted according to the instructions given in this manual 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x2). Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low monkey IL-8 levels. Such samples require further dilution, e.g., 1:10 (90 µL Assay Buffer + 10 µL Sample) in order to precisely quantitate the actual monkey IL-8 level.

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