ELISA Kit

Catalog # **KMC0041 (96 tests)**
KMC0042 (192 tests)

Mouse
IL-4

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Contents and Storage

Storage

Store at 2 to 8°C.

Contents

Reagents Provided	96 Test Kit	192 Test Kit
Ms IL-4 Standard, lyophilized, recombinant Ms IL-4. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle	2 bottles
Incubation Buffer. Contains 0.05% sodium azide; 12 mL per bottle.	1 bottle	1 bottle
Ms IL-4 High and Low Controls, recombinant Ms IL-4, lyophilized. Contains 0.1% sodium azide. Refer to vial label for reconstitution volume and range. Once reconstituted, aliquot and store at -20°C or below. Avoid repeated freeze-thaw cycles.	2 vials	2 vials
Ms IL-4 Antibody Coated Wells, 96 Well Plate.	1 plate	2 plates
Ms IL-4 Biotin Conjugate, (Biotin-labeled anti-IL-4). Contains 0.1% sodium azide; 6 mL per bottle.	1 bottle	2 bottles
Streptavidin-HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials
Streptavidin-HRP Diluent. Contains 3.3 mM thymol; 25 mL per bottle.	1 bottle	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle	1 bottle
Stabilized Chromogen, *Tetramethylbenzidine (TMB);* 25 mL per bottle.	1 bottle	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle	1 bottle
Plate Covers, adhesive strips.	3	4

Disposal Note

This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Purpose

The Invitrogen Mouse Interleukin-4 (Ms IL-4) ELISA is to be used for the quantitative determination of Ms IL-4 in mouse serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Ms IL-4.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Invitrogen Ms IL-4 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Ms IL-4 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Ms IL-4 content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated monoclonal second antibody.

During the first incubation, the Ms IL-4 antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Ms IL-4 present in the original specimen.

Background Information

Interleukin-4 (IL-4) is a 15-19 kDa glycoprotein produced by Th2 class $CD4^+$ T-lymphocytes and mast cells. IL-4 is the major down-regulator of IFN-γ in Th1 $CD4^+$ T-cells. IL-4 stimulates CD23 production, expression of surface IgM, LFA-1 and LFA-3 molecules, all of which mediate growth of B-cells and prepare them for production of IL-6 and TNF-α during activation of T-cells. IL-4 works reciprocally with IFN-γ to regulate the growth of macrophages, monocytes and granulocytes. In mice transplanted with tumorigenic cells, IL-4 has demonstrated the ability to block tumor cell proliferation. This is believed to be the result of IL-4-induced infiltration of eosinophils and macrophages to the site of the tumor. Clinical studies are ongoing in the use of IL-4 as a potential therapeutic for advanced forms of cancer, as well as other inflammatory diseases such as rheumatoid arthritis. Accurate determination of IL-4 levels would also be of value in monitoring the progress of allergic conditions such as asthma and anaphylaxis. There is no cross-species reactivity between human and mouse IL-4.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders
- 37ºC incubator

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. **Do not mix or interchange different reagent lots from various kit lots**.
9. Do not use reagents after the kit expiration date.
10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
11. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
13. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing

- **Incomplete washing will adversely affect the test outcome**. All washing must be performed with the *Wash Buffer Concentrate (25X*) provided.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the washing instructions carefully.

Preparation of Reagents

Preparing Controls

Reconstitute controls in deionized water. Refer to control vial label for instructions. There are two control ranges on the vial:

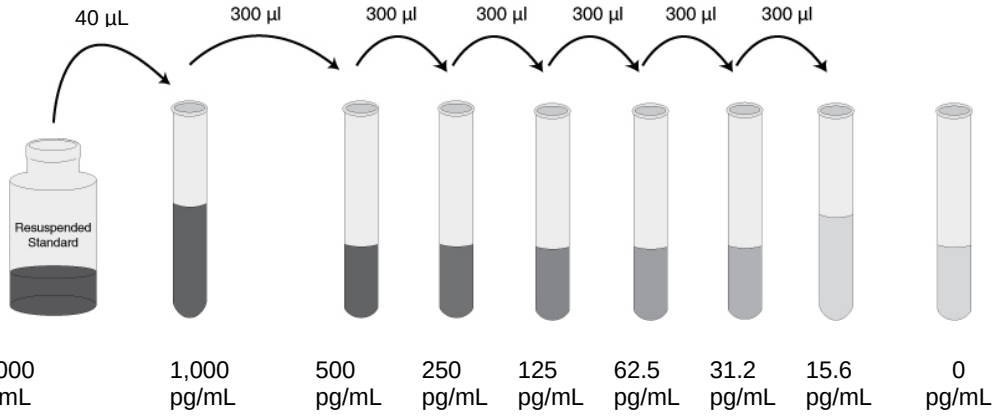
- The serum/plasma values for the control ranges on the vial label should be used to confirm assay validity for serum/plasma samples.
- The tissue culture values for the control ranges should be used to confirm assay validity for tissue culture supernatant samples. These control ranges were established using a standard curve reconstituted and serially diluted in RPMI supplemented with 10% fetal bovine serum.

Dilution of Standard

This assay has been calibrated against the WHO reference preparation 91/656 (NIBSC, Hertfordshire, UK, EN6 3QG). One microgram equals 10,000 units.

Note: Either glass or plastic tubes may be used for standard dilutions.

1. The standard should be reconstituted to 15,000 pg/mL with **either** *Standard Diluent Buffer* **or** tissue culture medium according to the following sample type:
   For serum or plasma samples: Reconstitute the standard using *Standard Diluent Buffer*. Serum or plasma samples should be quantified against a standard curve that is reconstituted and serially diluted in *Standard Diluent Buffer*.
   For tissue culture supernatant samples: Reconstitute the standard using the tissue culture medium used to culture samples. Tissue culture samples should be quantified against a standard curve reconstituted and serially diluted in the tissue culture medium used to culture samples.
2. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. It is recommended that the standard be used within 1 hour of reconstitution.
3. Add 0.040 mL of the reconstituted standard to a tube containing 0.560 mL *Standard Diluent Buffer* (for serum and plasma samples) or *Tissue Culture Medium* (for tissue culture samples). Label as 1000 pg/mL Ms IL-4. Mix.
4. Add 0.300 mL of *Standard Diluent Buffer* (for serum and plasma samples) or *Tissue Culture Medium* (for tissue culture samples) to each of 6 tubes labeled 500, 250, 125, 62.5, 31.2 and 15.6 pg/mL Ms IL-4.
5. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.



Preparing SAV-HRP

Note: Prepare within 15 minutes of usage. The *Streptavidin-HRP (100X)* is in 50% glycerol, which is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µl of this 100X concentrated solution with 1 ml of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.
2. Return the unused *Streptavidin-HRP (100X)* to the refrigerator.

# of 8-Well Strips	Volume of Streptavidin-HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

Dilution of Wash Buffer

1. Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 ml may be diluted up to 1.25 liters, 100 ml may be diluted up to 2.5 liters). Label as Working Wash Buffer.
2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 50 µL of the *Incubation Buffer* to the wells reserved for standards, controls, and unknowns.
3. Add 50 µL of the *Standard Diluent Buffer* (for serum and plasma samples) or *Tissue Culture Medium* (for tissue culture samples) to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
4. Add 50 µL of standards, samples or controls to the appropriate microtiter wells. See **Dilution of Standards**.
5. Pipette 50 µL of biotinylated *Ms IL-4 Biotin Conjugate* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
6. Cover plate with *plate cover* and incubate for **2 hours at 37°C**.
7. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing.**
8. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). Prepare the working dilution as described in **Preparing SAV-HRP**.
9. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.

10. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing.**
11. Add 100 µL of *Stabilized Chromogen* to each well. (Color of chromogen should be clear prior to adding it to the well). The liquid in the wells will begin to turn blue.
12. Incubate for **30 minutes at room temperature and in the dark**. ***Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceeds the limits of the instrument. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
13. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
14. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
15. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
16. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be diluted in *Standard Diluent Buffer* (serum of plasma) or *Tissue Culture Medium* (tissue culture samples) and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

Typical Data (Example)

The following data were obtained for the various standards over the range of 0 to 1000 pg/mL Ms IL-4.

Standard Ms IL-4 (pg/mL)	Optical Density (450 nm)
1000	2.888
500	1.943
250	1.234
125	0.716
62.5	0.380
31.2	0.211
15.6	0.124
0	0.023

Performance Characteristics

Sensitivity

The minimum detectable dose of Ms IL-4 is < 5 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Precision

1. Intra-Assay Precision

Samples of known Ms IL-4 concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	50.9	290.7	702.6
SD	2.3	9.8	42.3
%CV	4.5	3.4	6.0

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 42 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	52.1	290.1	704.9
SD	3.8	15.8	53.0
%CV	7.3	5.4	7.5

SD = Standard Deviation
CV = Coefficient of Variation

Linearity of Dilution

Mouse serum and tissue culture medium containing 10% fetal bovine serum were spiked with Ms IL-4 and serially diluted over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

	Serum			Cell Culture		
Dilution	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
neat	575	-	-	622	-	-
1/2	289	288	100	319	311	103
1/4	155	144	108	150	156	96
1/8	81	72	113	81	78	104
1/16	40	36	111	39	39	100
1/32	19	18	106	18	19	95
1/64	9	9	100	9	10	90

Recovery

The recovery of Ms IL-4 added to serum, plasma, or tissue culture medium is shown below.

Sample	Range	Average
Normal serum (n=16)	70-86%	77%
EDTA plasma (n=10)	68-87%	81%
Heparin plasma (n=10)	66-95%	76%
Citrate plasma (n=10)	70-98%	83%
Tissue culture medium (1% FBS) (n=9)	85-115%	101%
Tissue culture medium (10% FBS) (n=7)	101%-109%	106%

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Invitrogen Ms IL-4 kit. The following substances were tested and found to have no cross-activity: human IL-2, IL-3, IL-4, IL-5; mouse IL-1β, IL-2, IL-3, IL-6, IL-10, IL-12, IFN-γ, MCP-1, TNF-α; rat IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ, MCP-1, MIP-2, TNF-α.

High Dose Hook Effect

A sample spiked with 7.8 ng/mL of IL-4 gave an optical density reading higher than that obtained for the highest standard point.

Expected Values

Fifteen sera and fifteen plasma (EDTA) were evaluated in this assay. All samples measured <15.6 pg/mL (the lowest Ms IL-4 standard).

Mouse splenocytes were cultured under the following conditions and the culture supernatants were assayed for Ms IL-4 released.

Stimulation	**Concentration**
LPS (1 mg/mL) 24 hr	3 pg/mL
LPS (10 mg/mL) 24 hr	35 pg/mL
Con-A (5 mg/mL) 24 hr	100 pg/mL
Con-A (5 mg/mL) 48 hr	55 pg/mL
PMA (50 ng/mL), Ionophore (250 ng/mL) 6 hr	55 pg/mL

Limitations of the Procedure

Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Ms IL-4 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Appendix

Troubleshooting Guide

Elevated background	*Cause:* Insufficient washing and/or draining of wells after washing. Solution containing either biotin or SAV-HRP can elevate the background if residual is left in the well. *Solution:* Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid. *Cause:* Contamination of substrate solution with metal ions or oxidizing reagents. *Solution:* Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil. *Cause:* Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate. *Solution:* Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen. *Cause:* Incubation time is too long or incubation temperature is too high. *Solution:* Reduce incubation time and/or temperature.
Elevated sample/ standard ODs	*Cause:* Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly. *Solution*: Follow the protocol instructions regarding the dilution of the standard. *Cause:* Incorrect dilution of the SAV-HRP conjugate. *Solution:* Warm solution of SAV-HRP concentrate to room temperature, draw up slowly and wipe tip with kim-wipe to remove excess. Dilute ONLY in SAV diluent provided. *Cause*: Incubation times extended. *Solution:* Follow incubation times outlined in protocol. *Cause:* Incubations carried out at 37°C when RT is dictated. *Solution:* Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.
Poor standard curve	*Cause:* Improper preparation of standard stock solution. *Solution:* Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample. *Cause:* Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted. *Solution:* NEVER substitute any components from another kit. *Cause*: Errors in pipetting the standard or subsequent steps. *Solution*: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no color develops	*Cause:* Reagents not at RT (25 ± 2°C) at start of assay. *Solution:* Allow ALL reagents to warm to RT prior to commencing assay. *Cause:* Incorrect storage of components, e.g., not stored at 2 to 8°C. *Solution:* Store all components exactly as directed in protocol and on labels. *Cause:* Working SAV-HRP solution made up longer than 15 minutes before use in assay. *Solution:* Use the diluted SAV-HRP within 15 minutes of dilution. *Cause:* TMB solution lost activity. *Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. *Solution 2:* Avoid contact of the TMB solution with items containing metal ions. *Cause:* Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized. *Solution:* Please contact Technical Support for advice when using non-validated sample types. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.
Poor Precision	*Cause:* Errors in pipetting the standards, samples or subsequent steps. *Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip. *Cause:* Repetitive use of tips for several samples or different reagents. *Solution:* Use fresh tips for each sample or reagent transfer. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

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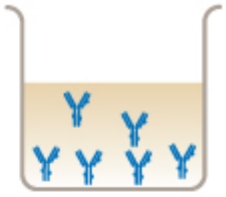
Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Mouse IL-4 Assay Summary

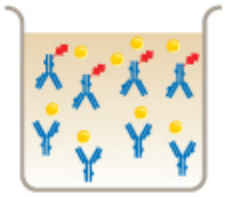
Add 50 µL Incubation buffer

Add 50 µL of standards, controls and samples



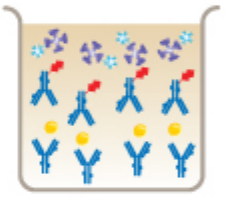
Add 50 µL of Biotin Conjugate
Incubate for 2 hr at 37°C

aspirate and wash 4x

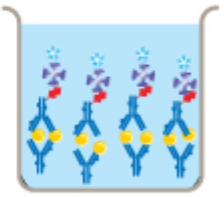


Incubate 100 µL of Streptavidin-HRP
Working Solution for 30 minutes at RT

aspirate and wash 4x



Incubate 100 µL of Stabilized Chromogen
for 30 minutes at RT



Add 100 µL of Stop Solution
Read at 450 nm

Total time: 3 hr