Human IL-10 ELISA Kit

Cat No. ELI-M-07-96
Size: 96 tests

MOLEQULE-ON®

Components	Quantity
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 Plate
Standard - lyophilized,2000 pg/ml upon reconstitution	2 Vials
Concentrated Biotin-Conjugated antibody (100X) - 120 ul/vial	1 Vial
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 Vial
Standard /sample Diluent - 16 ml/vial	1 Bottle
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 Bottle
Streptavidin-HRP Diluent - 16 ml/vial	1 Bottle
Wash Buffer Concentrate (20x) - 30 ml/vial	1 Bottle
Substrate Solution - 12 ml/vial	1 Bottle
Stop Solution - 12 ml/vial	1 Bottle
Plate Cover Seals	4 pieces

Reagent Preparations

Temperature returning - Bring all kit components and specimen to room temperature(20-25℃) before use.

Wash Buffer - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Standard\Specimen - Reconstitute the Standard with 1.0mL of deionized or distilled water. This reconstitution produces a stock solution of 2000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 850µL of Standard/Specimen Diluent into the 300pg/mL tube, and add 150µL stock solution of 2000pg/mL into it to get the high standard of 300pg/mL. Pipette 500µL of Standard/Specimen Diluent into the remaining tubes. Use the high standard of 300pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 300pg/mL standard serves as the high standard. The Standard/specimen Diluent serves as the zero standard (0pg/mL).

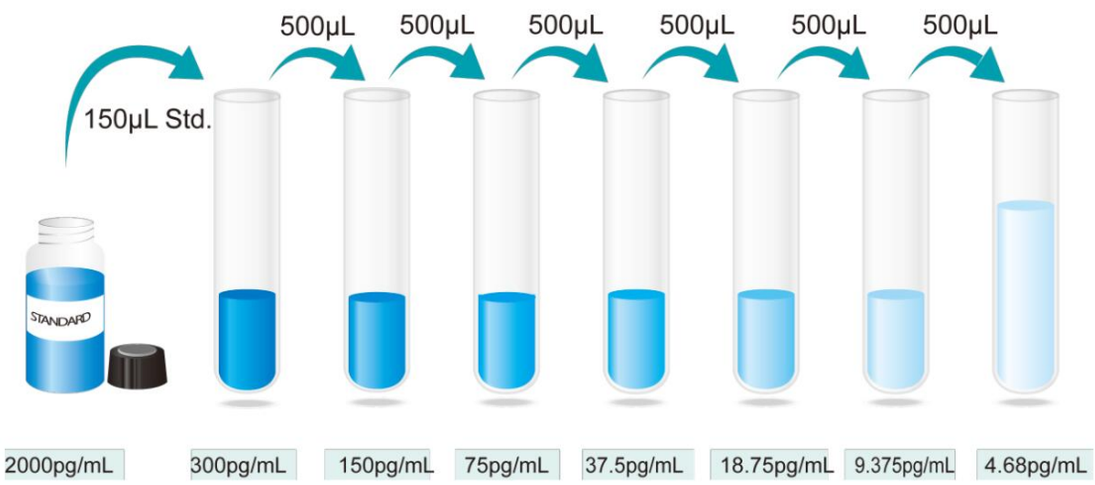
***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

Working solution of Biotin-Conjugate anti-human IL-10 antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**

Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**



Storage

All components of Human IL-10 ELISA Kit should be store at 2-8°C except reconstituted standard that should be store at -20°C.

Description

IL-10, also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine that is produced by T cells, NK cells, mast cells and macrophages. It is capable of inhibiting synthesis of pro-inflammatory cytokines like IFN-γ, IL-2, IL-3, TNF-α and GM-CSF made by cells such as macrophages and regulatory T-cells. IL-10 also displays potent abilities to suppress the antigen presentation capacity of antigen presenting cells. In addition, IL-10 promotes T cell tolerance by inhibiting tyrosine phosphorylation of CD28. IL-10 is an important negative regulator of the immune response, which allows for maintenance of pregnancy. In contrast, increased IL-10 levels contribute to persistent Leishmania major infections.

Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-10 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for IL-10 is added to detect the captured IL-10 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by Tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Sample Collection And Storage

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately for 15 minutes at 1000g(or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g(or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

Procedure

1. Prepare all reagents and working standards as directed in the previous sections.
2. 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. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8°C sealed tightly.
3. Add 100µl of standard or samples to each well and incubate for 90 minutes at 37°C.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µl) using a multichannel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100µl working solution of Biotin-Conjugate anti-human IL-10 antibody to each well, incubate for 60 minutes at 37°C.
6. Repeat step 4.
7. Add 100µl working solution of Streptavidin-HRP to each well, incubate for 30 minutes at 37°C.
8. Repeat step 4 with an additional wash.
9. Add 100µl of Substrate Solution to each well. Incubate for 15 minutes at 37°C. Avoid placing the plate in direct light.
10. Add 50µl Stop solution to each well. Gently tap the plate to ensure thorough mixing.
11. Read at 450nm within 30 minutes.

Technical Note

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

Calculation of Results

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the IL-10 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standared (pg/ml)	OD.	OD.	Average	Corrected
0	0.032	0.035	0.034	--------
4.68	0.085	0.081	0.083	0.050
9.375	0.129	0.125	0.127	0.094
18.75	0.259	0.257	0.258	0.225
37.5	0.551	0.536	0.544	0.510
75	0.980	1.010	0.995	0.962
150	2.025	2.016	2.021	1.987
300	3.177	3.172	3.175	3.141

Sensitivity, Specificity And Repeatability

SENSITIVITY: The minimum detectable dose was 2pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant human IL-10. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.
REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY： The recovery of IL-10 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

References

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