Rat IL-10 ELISA Kit

Catalog No. BSKR1014 (96 wells)
For Use with serum, plasma and cell culture supernatants

For Research Use Only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

Introductions	- 1 -
Principle of the Assay	- 1 -
Materials supplied	- 2 -
Storage	- 2 -
Materials Needed but Not Supplied	- 3 -
Precautions for Use	- 3 -
Sample Collection and Storage	- 4 -
Reagent Preparation	- 4 -
General ELISA Protocol	- 5 -
Assay Procedure Summary	- 6 -
Technical Hints	- 6 -
Calculation of Results	- 6 -
Performance Characteristics	- 7 -

Introductions

Interleukin-10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 α-helical cytokine family that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155. IL-10 is a pleiotropic cytokine that exerts immunosuppressive or immunostimulatory effects in a variety of cell types. In 1989, Fiorentino et al. found that the mouse Th2 cell line D10.G4.1 produced a new cytokine that inhibits the transcription of cytokine mRNA in Th1 cell line, called cytokine synthesis inhibitory factor (CSIF) and named IL-10 in the same year.

Mouse, rat and human IL-10 cDNA sequences each contain 178 amino acid residues and have an 18 amino acid signal peptide sequence. The mature IL-10 molecule is 160 amino acid residues, and the mouse and human IL-10 molecules contain 5 and 4 cysteine residues respectively. Their molecular weight ranges from 35-40 kDa. At the amino acid level, rat IL-10 has 85% and 74% homology to human and mouse IL-10 respectively. Rat and human IL-10 act on mouse-derived cells, whereas mouse IL-10 does not cross-react to human cells.

IL-10 mediates its biological activities through a heteromeric receptor complex composed of the type II cytokine receptor subunits IL-10 Rα and IL-10 Rβ. IL-10 Rα is a 110 kDa transmembrane glycoprotein that is expressed on lymphocytes, NK cells, macrophages, monocytes, astrocytes, intestinal epithelial cells, cytotrophoblasts, and activated hepatic stellate cells, while the 75 kDa transmembrane IL-10 Rβ is widely expressed. The IL-10 dimer binds to two IL-10 Rα chains, triggering recruitment of two IL-10 Rβ chains. IL-10 Rβ does not bind IL-10 directly but is required for signal transduction. IL-10 Rβ also associates with IL-20 Rα, IL-22 Rα1, or IL-28 Rα to form the receptor complexes for IL-22, IL-26, IL-28, and IL-29.

Antagonists of IL-10 may have anti-EB virus effects; IL-10 may become an anti-inflammatory treatment by promoting the expression of IL-1 Rα by monocytes. Animal experiments show that IL-10 can effectively prevent the death of mouse shock induced by LPS.

Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-10 has been pre-coated onto a microplate. Standard, control, or sample and the working solution of Biotin-Conjugate are pipetted into the wells. Following incubation and wash steps, any IL-10 present is bound by the immobilized antibody and the detection antibody specific for IL-10 is binds to the combination of capture antibody-IL-10 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of IL-10 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven IL-10 standard dilutions and IL-10 sample concentration determined.

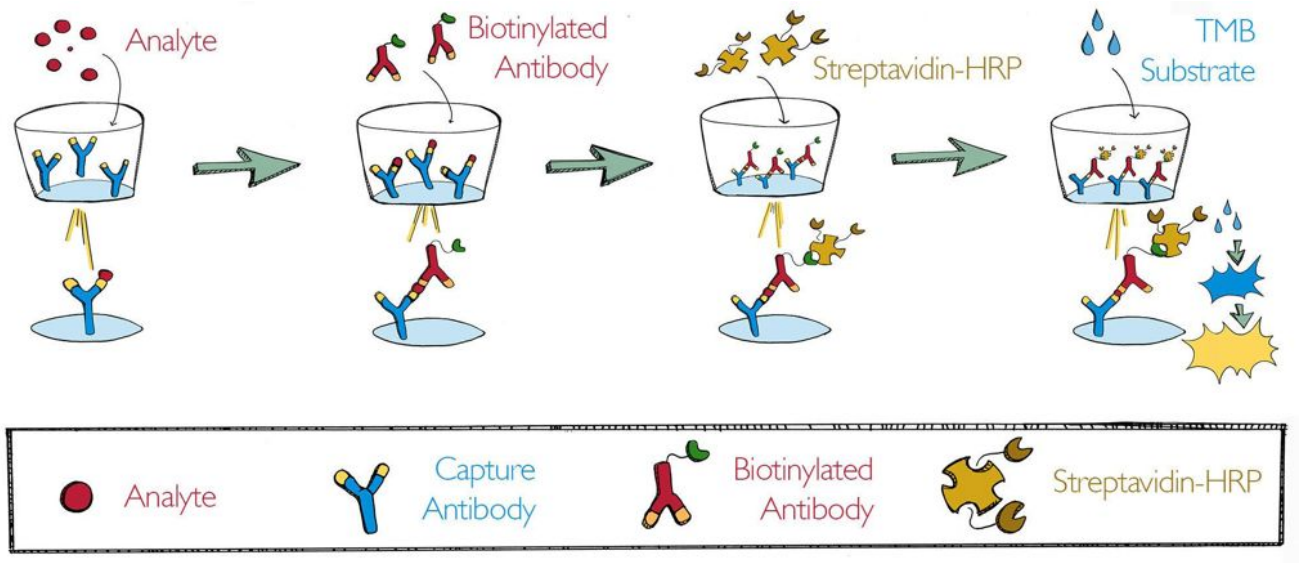

Figure 1. Schematic diagram of the assay

Materials supplied

Table 1. Kit Components

Kit Components	96 wells Quantity/Size
Aluminium pouches with a Microwell Plate coated with antibody to rat IL-10 (8×12)	1 plate
Rat IL-10 standard lyophilized, 2000pg/ml upon reconstitution	2 vials
Concentrated Biotin-Conjugate anti-rat IL-10 antibody	2 vials
Streptavidin-HRP solution	2 vials
standard /sample Diluent	1 bottle
Biotin-Conjugate antibody Diluent	1 bottle
Streptavidin-HRP Diluent	1 bottle
Wash Buffer Concentrate 20x (PBS with 1% Tween-20)	1 bottle
Substrate Solution	1 vial
Stop Solution	1 vial
Adhesive Films	4 pieces
Product data sheet	1 copy

Storage

Table 2. Storage of the kit

Unopened Kit	Store at 2 - 8°C. Do **NOT** use past kit expiration date**!**	
Opened/ Reconstituted	Standard /Sample Diluent	May be stored for up to 1 month at 2 - 8°C.**
	Concentrated Biotin-Conjugate	

Reagents	Streptavidin-HRP Solution	
	Biotin-Conjugate Antibody Diluent	
	Streptavidin-HRP Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	
	Stop Solution	
	Standard	Aliquot and store for up to 1 month at ≤20°C. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.**

**Provided this is within the expiration date of the kit.

Materials Needed but Not Supplied

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000µL.
3. 37℃ incubator.
4. Double-distilled water or deionized water.
5. Coordinate paper.
6. Graduated cylinder.

Precautions for Use

1. Store kit reagents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If

samples generate values higher than the highest standard, dilute the samples and repeat the assay.

12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

Sample Collection and Storage

1. **Cell Culture Supernatants** – Remove particulates by centrifugation.
2. **Serum** – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** – Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor). **Note: The normal rat serum or plasma samples are suggested to make a 1:2 dilution.**

Reagent Preparation

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** – Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL 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.
3. **Standard** – Reconstitute the standard with 1mL of standard /sample Diluent. This reconstitution produces a stock solution of 2000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500µL of standard/sample Diluent into the 1000 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2000 pg/mL standard serves as the high standard. The standard/ sample Diluent serves as the zero standard (0 pg/mL).

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

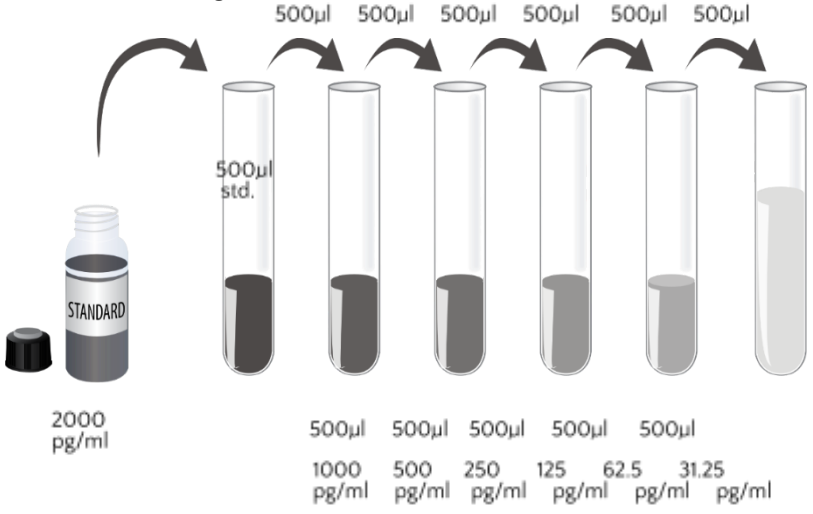

Figure 2. Preparation of IL-10 standard dilutions

4. Working solution of Biotin-Conjugate anti-rat 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.

5. 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.

General ELISA Protocol

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℃ sealed tightly.
3. Add 100µL of standard, control, or sample, per well, then add 50 µL of the working solution of Biotin-Conjugate to each well. Cover with the adhesive strip provided and incubate 2 hours at RT. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
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 squirt bottle, 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 100 µL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at RT. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for 10-20 minutes at RT. Avoid placing the plate in direct light.
8. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (optionally 630nm as the reference wavelength; 610-650nm is acceptable).

Assay Procedure Summary

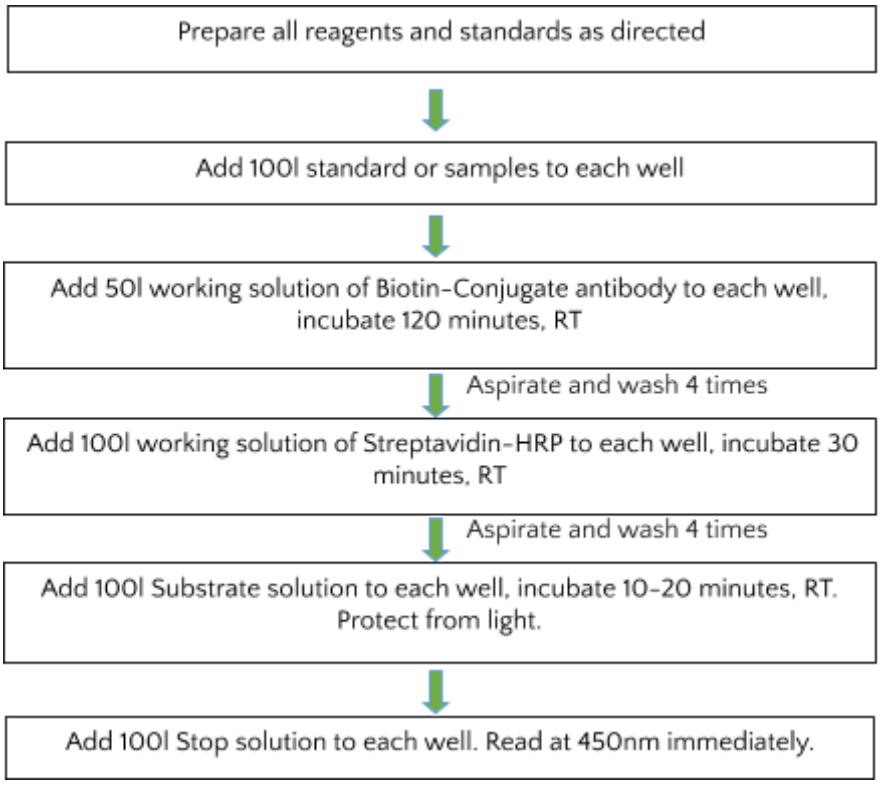

Figure 3. Assay procedure summary

Technical Hints

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. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. 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. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create 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.
3. 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.

4. This standard curve is provided **for demonstration only**. A standard curve should be generated for each set of samples assayed.

Table 3. Typical data using the IL-10 ELISA (Measuring wavelength:450nm, Reference wavelength:630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.098	0.095	0.097	——
31.25	0.209	0.206	0.208	0.201
62.5	0.275	0.280	0.278	0.261
125	0.389	0.397	0.393	0.380
250	0.621	0.628	0.625	0.608
500	1.020	1.028	1.024	1.028
1000	1.709	1.706	1.708	1.718
2000	2.512	2.503	2.508	2.506

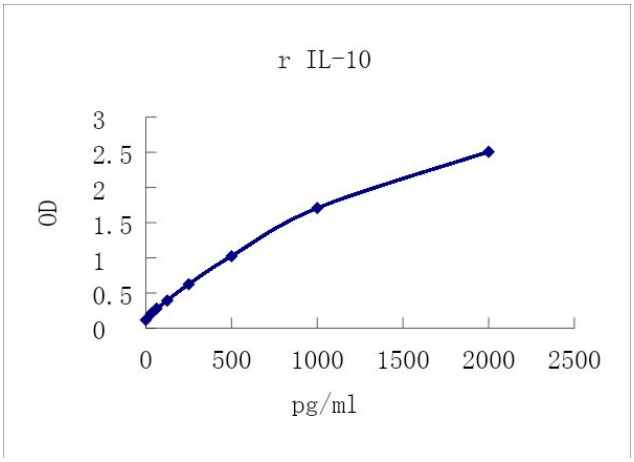

Figure 4. Representative standard curve for IL-10 ELISA.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Performance Characteristics

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

SENSITIVITY: The minimum detectable dose was 15pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Rat IL-10. The factors listed below were prepared at 50ng/ml in standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 4. Factors assayed for cross-reactivity

Recombinant rat	Recombinant human	Recombinant mouse
CINC-1	IL-10	IL-10 sR
GDNF	IL-10sR	
GM-CSF		
IFN- γ		
β -NGF		
PDGF-BB		
TNF- α		
IL-1 α		
IL-1 β		
IL-2		
IL-4		
IL-6		
IL-18		