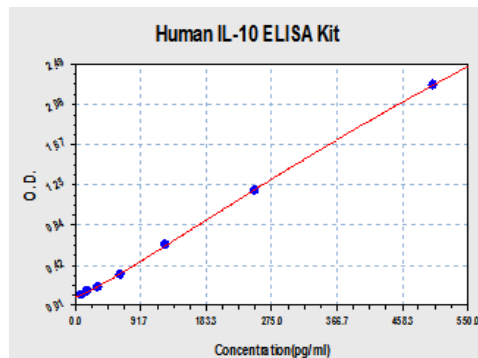


KIT COMPONENTS

Component	Amount
96-well plate pre-coated with anti-human IL-10 antibody	1 Plate
Protein Standard: Lyophilized recombinant human IL-10	2 tubes, 10 ng/tube
Sample Diluent Buffer	30 ml
Biotinylated Antibody (Anti-human IL-10)	130 µl (100x)
Antibody Diluent Buffer	12ml
Avidin-Biotin-Peroxidase Complex (ABC) Solution	130 µl (100x)
ABC Diluent Buffer	12 ml
Tetramethylbenzidine (TMB) Color Developing Agent	10 ml
TMB Stop Solution	10 ml

Storage

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.



X	pg/ml	0.0	7.8	15.6	31.3	62.5	125	250	500
Y	O.D.450	0.095	0.123	0.159	0.208	0.328	0.636	1.198	2.276

Figure 1: IL-10 Standard Curve. Using the human IL-10 ELISA Kit, O.D. data was graphed against IL-10 protein concentration. The TMB reaction was incubated at 37° C for 25 min.

BACKGROUND

Interleukin-10 (IL-10) is a pleiotropic cytokine produced by monocytes/macrophages, B cells, and various subsets of T cells including T helper type 2 (Th2), Th0 cells, and regulatory T (Treg) cells, to a lesser extent by Th1 cells, dendritic cells, mast cells and various tumor cell lines. Its main biological functions seem to limit and terminate the inflammatory responses, block the proinflammatory cytokine secretion and regulate the differentiation and proliferation of several immune competent cells such as T cells, B cells, natural killer cells, antigen-presenting cells, mast cells, and granulocytes. It plays important immunosuppressive and anti-inflammatory role in nature and therefore, it has been exploited therapeutically for the treatment of various autoimmune disorders such as inflammatory bowel disease, psoriasis and rheumatoid arthritis.¹ However, many studies suggest IL-10 also mediates immunostimulatory properties that help to eliminate infectious and noninfectious particles with limited inflammation. It has been shown that both human IL-10 and murine IL-10 exert immunostimulatory effects by up-regulating MHC class II expression on B lymphocytes, and inducing cytotoxic T-cell differentiation. These very different outcomes are believed to depend upon experimental conditions.² In addition, IL-10 overexpression was found in certain tumors and is considered to promote further tumor development. In contrast, a relative IL-10 deficiency has been observed and is regarded to be of pathophysiological relevance in certain inflammatory disorders characterized by a type 1 cytokine pattern such as psoriasis.³ However, systemic IL-10 release is a powerful tool of the central nervous system to prevent hyperinflammatory processes by activation of the neuro-endocrine axis following acute stress reactions.⁴ Human IL-10 binds as a 2-fold symmetric homodimer to a functional tetrameric complex of two receptors, consisting of two α- or R1 chains which bind to IL-10, and of two CRF2-4 chains (β- or R2) which initiate the IL-10-induced signal transduction events.

Reference

- Couper, K.N. et al: J. Immunol. 180:5771-7, 2008
- Asadullah, K. et al: Pharmacol. Rev. 55:241-69, 2003
- Asadullah, K. et al: Expert Opin. Investig. Drugs 9:95-102, 2000
- Platzer, C. et al: J. Neuroimmunol. 105:31-8, 2000

ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethylbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

NOTES:

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37°C for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.

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PROTOCOL

I. Plate Washing

Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of three washes.

II. Preparation of Test Samples

Test Sample Processing

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles

- **Cell culture supernate, tissue lysate or body fluids:** Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C.
- **Serum:** Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature or coat at 4°C overnight. Centrifuge at approximately 1000 X g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- **Plasma:** Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°.

Sample Dilution Guideline

Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follow

Target Protein Concentration Range	Sample Working Dilution	Sample Vol.	Diluent Buffer Vol.
5-50 ng/ml	1:100	1 µl	99 µl
500-5000 pg/ml	1:10	10 µl	90 µl
7.8-500 pg/ml	1:2	50 µl	50 µl
≤7.8 pg/ml	n/a	100µl	n/a

If samples will be assayed within 24 hours, store at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

III. Preparation of Reagents

Reconstitution of the Standard

The standard solutions should be prepared no more than 2 hours prior to the experiment. Two tubes of the standard are included in each kit. Use one tube for each experiment.

1. 10,000pg/ml of human IL-10 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
2. 500pg/ml of human IL-10 standard solution: Add 0.05 ml of the above 10ng/ml IL-10 standard solution into 0.95 ml sample diluent buffer and mix thoroughly.
3. 250pg/ml→7.8pg/ml of human IL-10 standard solutions: Label 6 Eppendorf tubes with 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml, 7.8pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 500pg/ml IL-10 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Preparation of Biotinylated Antibody Working Solution

The solution should be prepared no more than 2 hours prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Biotinylated antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

Preparation of the Avidin-Biotin-Peroxidase Complex (ABC) Working Solution

The solution should be prepared no more than 1 hour prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

IV. ELISA

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard IL-10 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of IL-10 amount in samples.

1. Aliquot 0.1ml per well of the 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml, 7.8pg/ml human IL-10 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human IL-10 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human IL-10 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 20-25 min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human IL-10 standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution

V. Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
 $O.D.450(Relative) = O.D.450(Reading) - O.D.450(Blank)$
- Plot the standard curve:
 Plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.
- The target protein concentration in samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.

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