Human IL10 ELISA Kit

Enzyme Immunoassay for the quantification of human Interleukin-10 (IL10) in human serum.

Catalog number: ARG80828

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Biological activities

Human interleukin-10 (IL-10) is a 19 kDA lymphokine produced by T helper lymphocytes,by monocytes, macrophages and B-lymphocytes. IL-10 was first characterized as a cytokine synthesis inhibitory factor (CSIF) able to inhibit cytokine synthesis by TH1 clones activated in the presence of antigen presenting cells. However, in the absence of monocytes, IL-10 directly inhibits the growth of T-cells triggered by immobilized anti-CD3 MoAb. This proliferation inhibition was found to be a result of specific inhibition of IL-2 production by the responding T-cells. In vitro, Il-10 is a very powerful inhibitor of monokines (including TNF-alpha, IL-1, IL-6 and IL-8) produced by LPS-activated monocytes and macrophages. The addition of IL-10 to B lymphocytes results in limited cell proliferation but most importantly in very high immunoglobulin production, a result of the transformation of B-cells into plasma cells. Finally, natural killer (NK) cells appear to be another target for the anti-inflammatory properties of IL-10. Indeed, recent data have shown that IL-10 can inhibit antigen induced IFN-gamma production by NK-cells by inhibiting not only production but also the stimulatory effects of IL-12 and TNF on IFN-gamma production.

Clinical application

So far, circulating levels of IL-10 have been found in serum of patients suffering of Non-Hodgkin's lymphoma, multiple myeloma, cerebral malaria or septic shock.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique. A highly specific IL-10 antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-antibody specific for IL-10 is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IL-10 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of IL-10 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C.
Standards 0-5 (0, 34, 82, 221, 756, 2450 pg/ml)	6 vials	4°C, lyophilized
Control Serum KS1 (168.62 pg/ml; range: 157-199 pg/ml)	1 vial	4°C, lyophilized
Control Serum KS2 (472 pg/ml; range: 455-601 pg/ml)	1 vial	4°C, lyophilized
HRP-antibody conjugate	6 ml (ready to use)	4°C
Specimen Diluent	3 vials (6 ml/each)	4°C, lyophilized

Incubation Buffer	11 ml (Ready to use)	4°C
200X Wash buffer	10 ml	4°C
Substrate buffer	3 X 21 ml (Ready to use)	4°C
TMB substrate concentrate	1 ml	4°C (Protect from light)
STOP solution	6 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm, 490 nm and optional reference wave length at 630 or 650 nm.
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 200X Wash buffer, incubation buffer and substrate buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.

- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C up to 2 months or at -70°C up to a year. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer.
- Standards: Reconstituted in 1 ml (each) distilled water.
- Controls: Reconstituted in 1 ml (each) distilled water.
- Specimen Diluent: Reconstitute specimen diluent in 6 ml (each) distilled water.

  Note: After reconstitution, standards, controls and specimen Diluent are stable for 4 days at 2 to 8°C. For longer storage periods, aliquots and store at-20°C for up to 2 months. Avoid repeated freeze thaw cycles.
- TMB Reagent: Dilute 0.2 ml TMB substrate concentrate into 1 vial of substrate buffer. Extemporaneous preparation is recommended. The freshly prepared revelation solution is stable, before use, for maximum 15 minutes at room temperature and must be discarded afterwards.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of incubation buffer into all wells.
3. Add 100 µl of standards, controls and samples into respective wells.
4. Cover wells and incubate for 2 hours at RT with shaking at ≥ 600 rpm.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (400 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl of specimen diluent and then 50 µl of HRP-antibody conjugate into each well. Cover wells and incubate 2 hours at RT with shaking at ≥ 600 rpm.
7. Aspirate and wash each well as step 5.
8. Add 200 µl of freshly prepared TMB Reagent to each well. Incubate for 30 minutes at room temperature in dark with shaking at ≥ 600 rpm.
9. Add 50 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450 nm and 490 nm immediately. (Using 630 nm or 650 nm as optional reference wave length)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. The principle of polychromatic data processing is as follows:

   X_{450} = OD at 450 nm

   X_{490} = OD at 490 nm

   Using a standard unweighted linear regression, the parameters A & B are calculated: $Y = A*X + B$

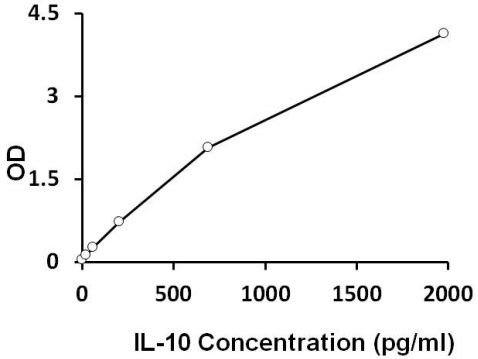
   If $X_{450} < 3$ OD units, then X calculated = X_{450}

   If $X_{450} > 3$ OD units, then X calculated = $(X_{490}-B)/A$

   A 4-parameter logistic curve fitting is used to build up the calibration curve.
5. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of IL-10 range was 1.6 pg/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.25% and inter-assay precision was 2.75%.

Specificity

No significant cross-reaction was observed in presence of 50 ng of IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, TNF-α, TNF-β, IFN-α, IFN-β, IFN-γ, TGF-β, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, RANTES, PF-4,

βTG, GRO, IP-10 and SCF. This IL-10 assay is specific for human natural and recombinant IL-10.

A very low level (<0.2%) of cross-reaction was observed with BRCF1 (viral IL-10) at a concentration of 70000 pg/ml. BCRF1 gave a signal corresponding to 134 pg/ml of IL-10.

Recovery

93-100%