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Product Information

Human IL-10 R α ELISA Kit
for serum, plasma, cell culture supernatant and urine

Catalog Number **RAB0248**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Human IL-10 R α ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human IL-10 R α in serum, plasma (human IL-10 R α concentration is low in normal serum/plasma and may not be detected in this assay), cell culture supernatants, and urine. This assay employs an antibody specific for human IL-10 R α coated on a 96 well plate. Standards and samples are pipetted into the wells and IL-10 R α present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IL-10 R α antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-10 R α bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. Human IL-10 R α Antibody-coated ELISA Plate (Item A) - RAB0248A: 96 wells (12 strips × 8 wells) coated with anti-human IL-10 R α.
2. 20x Wash Buffer (Item B) - RABWASH4: 25 mL of 20x concentrated solution.
3. Lyophilized Human IL-10 R α Protein Standard (Item C) - RAB0248C: 2 vials of recombinant human IL-10 R α.
4. ELISA 1x Assay/Sample Diluent Buffer C (Item L) - RABELADC: 30 mL diluent buffer. For Standard/ Sample (serum/plasma) diluent.
5. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB: 15 mL of 5x concentrated buffer. For Standard/Sample (cell culture medium/urine) diluent.
6. Biotinylated Human IL-10 R α Detection Antibody (Item F) - RAB0248D: 2 vials of biotinylated anti-human IL-10 R α (each vial is enough to assay half microplate).
7. HRP-Streptavidin (Item G) - RABHRP5: 200 µL of 500x concentrated HRP-conjugated streptavidin.
8. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3: 12 mL of 3,3′,5,5′-tetra-methylbenzidine (TMB) in buffer solution.
9. ELISA Stop Solution (Item I) - RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µL to 1 mL volumes
3. Adjustable 1-25 mL pipettes for reagent preparation
4. 100 mL and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis
8. Tubes to prepare standard or sample dilutions

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1. Bring all reagents and samples to room temperature (18–25 °C) before use.

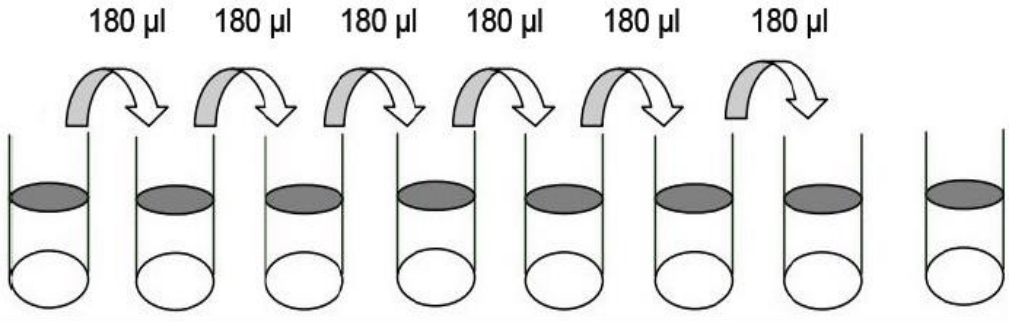
2. Sample dilution: Assay Diluent C (Item L) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of cell culture supernatants/urine. The suggested dilution for serum/plasma is 2-fold.

   Note: Levels of the IL-10 R alpha may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

4. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 µL of Assay Diluent C (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernatants/urine) into Item C vial to prepare a 500 ng/mL standard. **Dissolve the powder thoroughly by a gentle mix.** Pipette 270 µL of Assay Diluent C or 1x Assay Diluent B into each tube. Use the 500 ng/mL standard solution to produce a dilution series (see Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent C or 1x Assay Diluent B serves as the zero standard (0 ng/mL).

Figure 1.
Dilution Series for Standards



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Zero Standard
Diluent volume	Item C + 400 µl	270 µl	270 µl	270 µl	270 µl	270 µl	270 µl	270 µl
Conc.	500 ng/ml	200 ng/ml	80 ng/ml	32 ng/ml	12.8 ng/ml	5.12 ng/ml	2.05 ng/ml	0 ng/ml

5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in Procedure, step 5.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 500-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin concentrate into a tube with 10 mL of 1x Assay Diluent B to prepare a final 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Storage/Stability
Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8 well strips as appropriate for the experiment.

3. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking.

4. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multichannel pipette or autowasher. 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 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

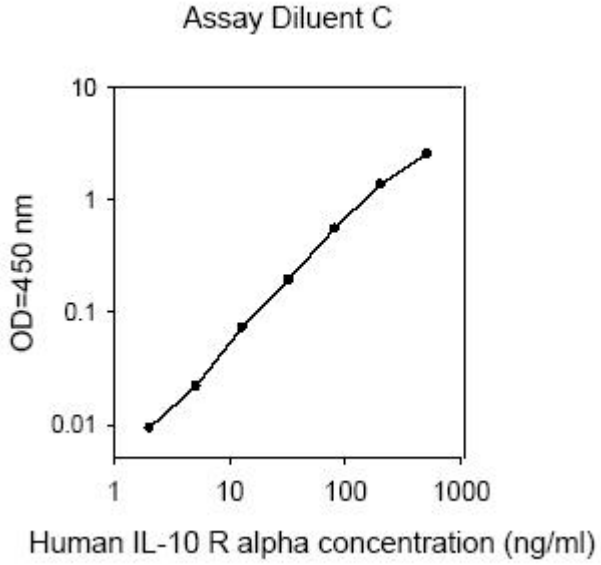
Results

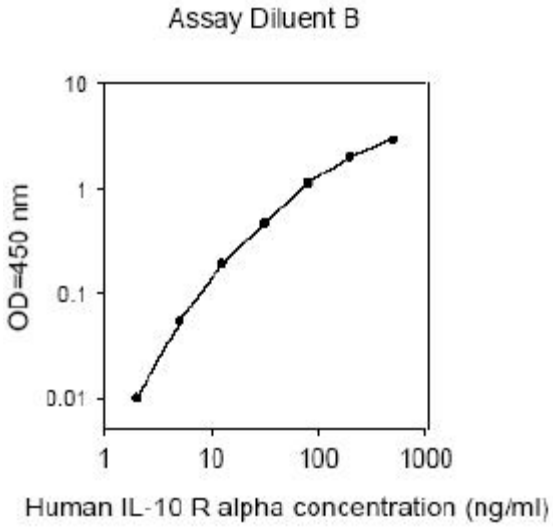
Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.





Product Profile

Sensitivity: The minimum detectable dose of Human IL-10 R α was determined to be 2.5 ng/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Reproducibility:

Intra-Assay: CV <10%

Inter-Assay: CV <12%

Spiking & Recovery: Recovery was determined by spiking various levels of human IL-10 R α into human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	89.97	83-96
Plasma	89.96	80-95
Cell culture media	99.67	90-108

Linearity:

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	86.47	104.4	97.57
	Range (%)	75-94	95-113	89-105
1:4	Average % of Expected	75.67	74.76	76.49
	Range (%)	67-84	67-83	68-85

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin, BDNF, BLC, CNTF, ENA-78, FGF-4, IL-1alpha, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, FGF-4, FGF-6, FGF-7, G-CSF, GDNF, GM-CSF, IFN-gamma, IGFBP-2, IGFBP-3, IGFBP-4, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIF, MIG, MIP-1alpha, MIP-1beta, MIP-1delta, PARC, PDGF,RANTES, SCF,SDF-1alpha, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, and VEGF.

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 3 may be done over night at 4 °C with gentle shaking. Note: may increase overall signals including background.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Add stop solution to each well before reading plate

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