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IL-7 (Human) ELISA Kit

(Catalog # K4154-100; 100 assays; Store at 4°C)

I. Introduction:

IL-7 is a hematopoietic growth factor capable of stimulating the proliferation of lymphoid progenitors. It is important for proliferation during certain stages of B-cell maturation, T and NK cell survival, development and homeostasis. IL-7 is a cytokine for B and T cell development. This cytokine can be produced locally by intestinal epithelial and epithelial goblet cells, and may serve as a regulatory factor for intestinal mucosal lymphocytes. The design of this assay is based on a sandwich ELISA. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to human IL-7. Samples are pipetted into these wells. The assay will recognize native and recombinant human IL-7.

II. Application:

Determine the quantity of IL-7 in human serum, human plasma, cell lysate, culture supernatants and buffered solution.

III. Specificity:

Human

IV. Sample Type:

Serum, plasma, cell lysate, culture supernatants and buffered solution

V. Kit Contents:

Components	K4154-100	Part No.
Plate coated with human IL-7 antibody	8-well strips x 12	K4154-100-1
Wash Buffer (20X)	25 ml x 2	K4154-100-2
Standard Protein (lyophilized)	1 vial	K4154-100-3
Standard/Sample Dilution Buffer	25 ml	K4154-100-4
Secondary Antibody (lyophilized)	1 vial	K4154-100-5
Streptavidin HRP (100X)	150 µl	K4154-100-6
Secondary antibody/ streptavidin HRP Dilution Buffer	25 ml	K4154-100-7
Substrate (TMB)	15 ml	K4154-100-8
Stop Solution	15 ml	K4154-100-9
Plate sealers (plastic film)	2	K4154-100-10

VI. User Supplied Reagents and Equipment:

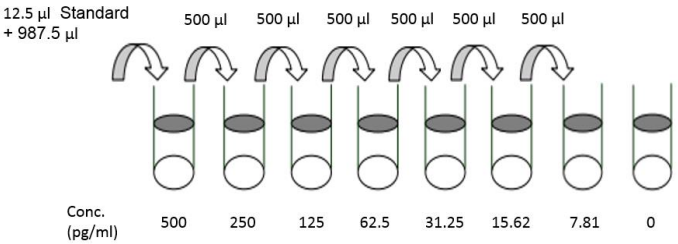
- Microtiterplate reader at 450 nm
- Calibrated precision single and multi-channel pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Vortex mixer
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

VII. Storage and Handling:

All kit components of this kit are stable at 2 to 8°C. Any unused reconstituted standard should be discarded or frozen at -70°C. Standard can be frozen and thawed one time only without loss of immunoreactivity.

VIII. Reagent and Sample Preparation and Storage Conditions:

- Bring all reagents and samples to room temperature before use.
- Wash Buffer 10X has to be diluted with deionized water 1:20 before use (e.g. 0.5 ml Wash Buffer 10X + 9.5 ml water) to obtain Wash Buffer 1X.
- Secondary Antibody
  - 100X secondary antibody solution can be made by adding 150 µl Secondary antibody/Streptavidin HRP dilution buffer in the vial.
  - Mix 20 µl Secondary Antibody concentrated solution (100X) + 1.98 ml Secondary antibody/ Streptavidin HRP dilution buffer.
  - Store unused Secondary Antibody concentrated solution in 4°C.
- Mix 20 µl Streptavidin HRP (100X) + 1.98 ml Secondary antibody/ Streptavidin HRP dilution buffer. Store unused Streptavidin HRP (100X) in 4°C
- Human IL-7 Standard has to be reconstituted with 1 ml of Standard/Sample Dilution Buffer to make the 40 ng/ml standard stock solution. Allow solution to sit at RT for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting. Dilute the standard protein concentrate (STD) (500 pg/ml) in Standard/Sample Dilution Buffer.
  - A seven-point standard curve using 2-fold serial dilutions in Standard/Sample Dilution Buffer is recommended. Standard/Sample Dilution Buffer serves as the zero standard (0 pg/ml).



FOR RESEARCH USE ONLY! Not to be used on humans.

- Sample Preparation:
  - Plasma: Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.
  - Urine: Aseptically collect the urine of the day, voided directly into a sterile container. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze/thaw cycles.
  - Serum and plasma require at least 16 fold dilution in the Standard/Sample Dilution Buffer.

IX. Assay Protocol:

** Bring all reagents and samples to room temperature before use. A standard curve must be run with each assay.

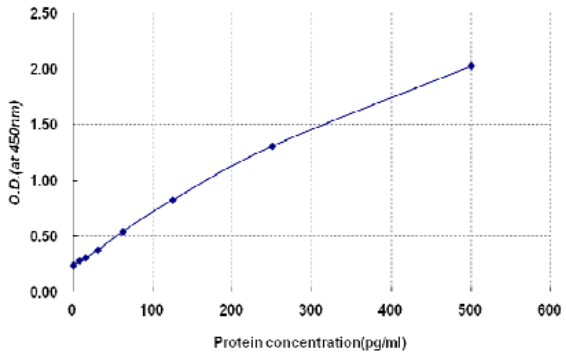
1. Determine the number of 12-well strips needed for the assay and insert them in the frame for current use. The extra strips should be resealed in the foil pouch bag and stored at 4°C.
   NOTE: Remaining strips and frame can be stored at 4°C for up to 1 month.
2. Add 100 µl of the different **standards** into the appropriate wells in duplicate. At the same time, add 100 µl of diluted serum, plasma, urine, cell culture supernatant **samples** in duplicate to the wells. Cover the plate with plate sealer and incubate for 2 hour at 37°C.
3. Aspirate the coated wells and soak wells with 300 µl **Wash Buffer 1X** for 1-3 minutes using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
4. Add 100 µl **Working Secondary Antibody Solution** to each well. Cover the plate with plate sealer and incubate for 1 hour at 37°C.
5. Aspirate the coated wells and soak wells with 300 µl **Wash Buffer** 1X for 1-3 minutes using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
6. Add 100 µl **Working Streptavidin HRP Solution** to each well. Cover the plate with plate sealer and incubate for 30 minutes at 37°C.
7. Aspirate the coated wells and soak wells with 300 µl **Wash Buffer** 1X for 1-3 minutes using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
8. Add 100 µl **TMB Substrate** to each well. Allow the color reaction to develop at room temperature for 10-15 minutes depending on the limitation of the limitation of microtiter plate reader.
9. Stop the reaction by adding 100 µl of **Stop Solution**. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
10. Measure the OD at 450 nm in an ELISA reader within 20 minutes.

X. Calculation of Results:

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding IL-7 concentration on the horizontal (X) axis (see Typical Data).
- Calculate the IL-7 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human IL-7 in the samples.

XI. Typical Data:

The following data are obtained using the different concentrations of standard as described in this protocol:



XII. RELATED PRODUCTS:

- IL-7 Antibody (Cat. No. 5103-50)
- IL-7 Antibody (Cat. No. 5148-100)
- Human CellExp™ IL-7, Human Recombinant (7274-10)
- IL-7, human recombinant (Cat. No. 4146-10, -50, -1000)
- IL-7, murine recombinant (Cat. No. 4147-10, -50, -1000)
- IL-7, rat recombinant (Cat. No. 4148-10, -50, -1000)

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