

IL-5 (Human) ELISA Kit

02/16

(Catalog # K4156-100, 100 assays, Store at 4°C)

I. Introduction:

Interleukin-5 (IL-5) is also known as eosinophil differentiation factor (EDF). It is a potential candidate gene in the pathogenesis of asthma, as it is the main cytokine controlling eosinophil activity and eosinophils are pivotal in the development of airway inflammation. IL-5 is also a factor that induces terminal differentiation of late-developing B-cells to immunoglobulin secreting cells. The predicted amino acid sequence of 134 amino acids is identical with that recently reported for human interleukin-5 but shows no significant homology with other known hemopoietic growth regulators. IL-5 is a lineage-specific hematopoietic growth factor that stimulates the production of eosinophils and eosinophil colonies from normal human bone marrow cells.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of human IL-5.

III. Specificity:

The capture antibody provided in this kit recognizes human IL-5.

IV. Sample Type:

Human serum, plasma, cell lysate, culture supernatants and buffered solution.

V. Kit Contents:

Components	K4156-100	Part No.
Microplate: 96 wells, coated with anti-human IL-5	1	K4156-100-1
Wash Buffer (20X)	25 ml x 2	K4156-100-2
Standard Protein (Lyophilized)	1 vials	K4156-100-3
Standard/Sample Dilution Buffer	25 ml	K4156-100-4
Secondary Antibody (Lyophilized)	1	K4156-100-5
Streptavidin HRP(X100)	150 µl	K4156-100-6
Secondary antibody/ streptavidin HRP Dilution Buffer	25 ml	K4156-100-7
Substrate (TMB)	15 ml	K4156-100-8
Stop Solution	15 ml	K4156-100-9
Plate sealers	2	K4156-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions

VII. Storage and Handling:

The entire kit may be stored at 4°C for up to 1 year from the date of shipment. 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 Preparation:

** Bring all reagents and samples to room temperature before use.

Reagent Dilution:

1. **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. Label as "Working Secondary antibody Solution". (Return the unused Secondary Antibody concentrated solution to 4°C.)
2. **Streptavidin HRP:** Mix 20 µl Streptavidin HRP concentrated solution (100X) + 1.98 ml Secondary antibody/Streptavidin HRP dilution buffer. (Sufficient for one 16-well strip, prepare more if needed)
3. **Washing buffer:** Mix 0.5 volume Wash buffer concentrate solution (20X) + 9.5 volumes of deionized water. (Store both the concentrated and the Working Washing Solution in 4°C.)

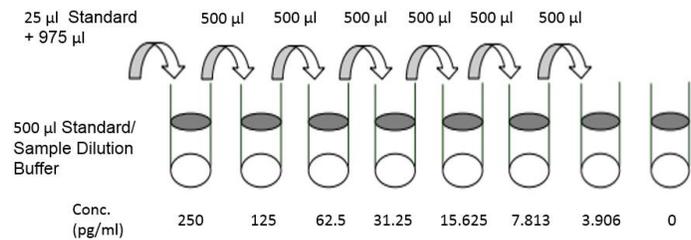
Standard Preparation:

- Reconstitute the lyophilized Human IL-5 standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 10 ng/ml standard stock solution.

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- Allow solution to sit at room temperature for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting.
- Prepare 1 ml of 250 pg/ml top standard by adding 25 μ l of the above stock solution in 975 μ l of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 250, 125, 62.5, 31.25, 15.625, 7.81, 3.906, 0 pg/ml



Sample Preparation:

- Plasma or Serum: blood may be drawn into tubes containing sodium citrate or heparin, EDTA. The serum or plasma should be separated from the coagulated or packed cells by centrifugation. Specimens may be stored at 4°C if the assay shall be run within one week. Keep samples at -20°C for longer storage. Avoid repeated freeze/thawing cycle.
- Serum and plasma samples require approximate 20 fold dilution in the Standard/Sample Dilution Buffer.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature before use.

It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Add 100 μ l of each **standard** and **samples** into appropriate wells. Cover well and incubate for 2 hours at 37°C.
3. Discard the solution and wash 3 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (300 μ l) using a multi-channel Pipette or autowasher. Let soak for 1 to 3 minutes and then all residual wash-liquid must be drained from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Pipette 100 μ l of Working **Secondary Antibody Solution** into each well. Incubate for 1 hour at 37°C. Discard the solution. Wash the wells as step 3.
5. Add 100 μ l working **Streptavidin HRP Solution** to each well. Cover well and incubate for 30 hours at 37°C. Discard the solution. Wash the wells as step 3.
6. Add 100 μ l of **Substrate** to each well. Cover well and incubate for 5-10 minutes at room temperature.
7. Add 100 μ l of **Stop Solution** to each well. Read result at 450 nm within 20 minutes.

X. CALCULATION:

- 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 absorbance on the vertical (Y) axis vs. the corresponding IL-5 concentration on the horizontal (X) axis.
- Calculate the IL-5 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.

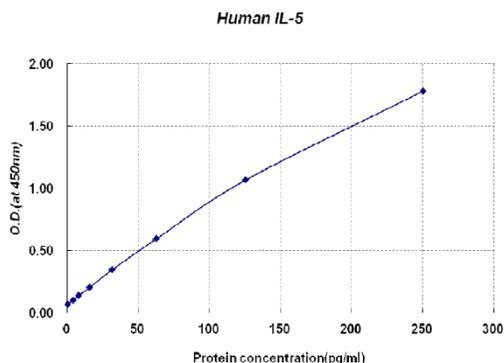


Figure: Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

XI. RELATED PRODUCTS:

- IL-5, human recombinant (Cat. No. 4140-10, -1000)
- IL-5, murine recombinant (Cat. No. 4141-10, -1000)
- IL-5, rat recombinant (Cat. No. 4142-10, -1000)

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