Product Manual

IL-5 (Human) ELISA Kit

(IL-5, Interleukin-5, B Cell Differentiation Factor I, T-cell Replacing Factor, TRF)

Catalog #: ENZ-KIT139

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

Interleukin-5 (IL-5) is also known as eosinophil differentiation factor (EDF). IL-5 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.[1] 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.[2] IL-5 is a lineage-specific hematopoietic growth factor that stimulates the production of eosinophils and eosinophil colonies from normal human bone marrow cells. IL-5 of human and mouse share 70% amino acid sequence homology.

INTENDED USE

The IL-5 (human) ELISA kit is a sandwich ELISA for the quantitative detection of human IL-5 in cell culture supernatants, serum, plasma (heparin, EDTA) and urine.

PRINCIPLE OF THE ASSAY

The IL-5 ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for IL-5 has been pre-coated onto 96-well plates. Standards and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for IL-5 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human IL-5 amount of sample captured in plate.

KIT COMPONENTS

1. 96-well plate pre-coated with anti-IL-5, Human
2. Lyophilized recombinant IL-5, Human standard, 10 ng/tube, 2 each
3. Anti-human IL-5 (Biotin), 130µl (1:100 dilution)
4. Avidin-Biotin-Peroxidase Complex (ABC), 130µl (1:100 dilution)
5. Sample diluent buffer, 30 ml
6. Antibody diluent buffer, 12 ml
7. ABC diluent buffer, 12 ml
8. TMB color developing agent, 10 ml
9. TMB stop solution, 10 ml

Avoid freeze / thaw cycles

STORAGE AND HANDLING

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

OTHER MATERIALS NEEDED

- Microplate reader in standard size
- Automated plate washer
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection
- Clean tubes and Eppendorf tubes
- Washing buffer (neutral PBS or TBS)

Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g sodium chloride; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01M PBS: Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

APPLICATION NOTES

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color Developing agent is colorless and transparent. Before using, please contact us if it is not the case.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Duplicate well assay is recommended for both standard and sample testing.
5. Don’t let 96-well plate dry, for dry plate will inactivate active components on plate.
6. Don’t reuse tips and tubes to avoid cross contamination.
7. To avoid using the reagents from different batches together.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

SAMPLE PREPARATION AND STORAGE

Store samples to be assayed within 24 hours at 4°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants:

Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

Serum*:*

Allow the serum to clot in a serum separator tube (about 4 hours) at RT. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

Plasma*:*

Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 x g within 30 minutes of collection.

Assay immediately or aliquot and store samples at -20°C.

Urine*:*

Aseptically collect the first urine of the day, micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

Note*:* The sample with hyperlipoidemia and hemolyticus is not suitable for this kit.

SAMPLE DILUTION GUIDELINE

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear range in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluent buffer.

a. High target protein concentration (5-50ng/ml). The working dilution is 1:100. (i.e. Add 1µl sample into 99µl sample diluent buffer)

b. Medium target protein concentration (500-5000 pg/ml). The working dilution is 1:10. (i.e. Add 10µl sample into 90µl sample diluent buffer)

c. Low target protein concentration (7.8-500 pg/ml). The working dilution is 1:2. (i.e. Add 50µl sample to 50µl sample diluent buffer).

d. Very low target protein concentration (≤7.8 pg/ml). No dilution necessary, or the working dilution is 1:2.

REAGENT PREPARATION AND STORAGE

Reconstitution of the IL-5 Standard

IL-5 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of IL-5 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

a. 10,000 pg/ml of human IL-5 standard solution: Add 1ml sample diluent buffer into one tube, keep the tube at RT for 10 min and mix thoroughly.

b. 500pg/ml of human IL-5 standard solution: Add 0.05 ml of the above 10 ng/ml IL-5 standard solution into 0.95 ml sample diluent buffer and mix thoroughly.

c. 250pg/ml → 7.8pg/ml of human IL-5 standard solutions: Label 6 Eppendorf tubes with 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml, 15.6 pg/ml, 7.8 pg/ml respectively. Aliquot 0.3ml of the sample diluent buffer into each tube. Add 0.3ml of the above 500pg/ml IL-5 standard solution into 1st tube and mix. Transfer 0.3ml from 1st tube to 2nd tube and mix. Transfer 0.3ml from 2nd tube to 3rd tube and mix, and so on.

***Note**:* The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

Preparation of Anti-human IL-5 (Biotin) antibody working solution

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

a. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

b. Biotinylated anti-human IL-5 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1µl Biotinylated anti-human IL-5 antibody to 99µl antibody diluent buffer.)

Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution

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

a. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

b. Avidin-Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly (i.e. Add 1µl ABC to 99µl ABC diluent buffer).

ASSAY PROCEDURE

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard IL-5 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of IL-5 amount in samples.

1. Aliquot 100µl per well of the 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml, 15.6 pg/ml, 7.8 pg/ml human IL-5 standard solutions into the pre-coated 96-well plate. Add 100µl of the sample diluent buffer into the control well (Zero well). Add 0.1 ml of each properly diluted sample of human cell culture supernatants, serum, plasma (heparin, EDTA) or urine to each empty well. See “Sample Dilution Guideline” above for details. It is recommended that each human IL-5 standard solution and each sample be measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 minutes.
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 100µl of biotinylated anti-human IL-5 antibody working solution into each well and incubate the plate at 37°C for 60 minutes.

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 0.3ml 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 100µl of prepared ABC working solution into each well and incubate the plate at 37°C for 30 minutes.
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 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 20-25 minutes (*Note:* For reference only, the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the four most concentrated human IL-5 standard solutions; the other wells show no obvious color).
9. Add 100µl of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human IL-5 concentration of the samples can be interpolated from the standard curve.

Note*:* If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

TYPICAL DATA

(TMB reaction incubate at 37°C for 20 min)

Concentration (pg/ml) O.D.

Concentration (pg/ml)	O.D.
0.0	0.003
7.8	0.036
15.6	0.075
31.3	0.134
62.5	0.316
125	0.737
250	1.319
500	2.209

***Range**:* 7.8-500 pg/ml

***Sensitivity**:* < 2 pg/ml

***Specificity**:* Natural and recombinant human IL-5

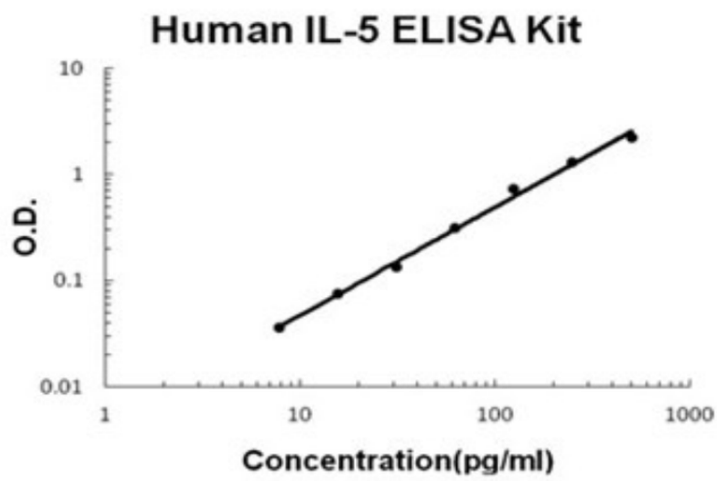
***Cross-reactivity**:* No detectable cross-reactivity with other relevant proteins

ASSAY SUMMARY

1. Add samples and standards and incubate the plate at 37°C for 90 minutes. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 minutes. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 minutes. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 25-30 minutes.
5. Add TMB stop solution and read.

IMAGES

Fig. 1. Typical standard curve (shown for reference only).



MPORTANT NOTE

This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.

STORAGE

4°C/-20°C

SHIPPING:

Blue Ice

REFERENCES:

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2. Campbell, H.D., et al., Molecular cloning, nucleotide sequence, and expression of the gene encoding human eosinophil differentiation factor (interleukin-5). Proc. Nat. Acad. Sci. 84: 6629-6633 (1987).
3. Le Beau, M.M., et al., Interleukin-4 and interleukin-5 map to human chromosome 5 in a region encoding growth factors and receptors and are deleted in myeloid leukemias with a del(5q). Blood 73: 647-650 (1989).

NOTES

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