IL-8 (Human) ELISA Kit

rev 11/21

(Catalog # K4169-100, 96 assays, Store at 4 °C)

I. Introduction:

IL-8 is a chemotactic factor that attracts neutrophils, basophils, and T-cells, but not monocytes. It is also involved in neutrophil activation. It is released from several cell types in response to an inflammatory stimulus. **BioVision's IL-8 ELISA Kit** is a sandwich ELISA assay for the quantitative measurement of human IL-8 in serum, plasma, tissue homogenates and other biological fluids. Capture antibody was pre-coated onto the 96- well plates and the biotin conjugated antibody was used as the detection antibody. The standards, test samples and biotin conjugated detection antibody are added to the wells subsequently followed by washing with wash buffer. HRP-Streptavidin was then added and unbound conjugates were washed away with wash buffer. TMB substrate was used to visualize the 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 color is proportional to the Human IL-8 amount of sample captured in plate.

II. Application & Key Features:

- This ELISA kit is used for *in vitro* quantitative determination of Human IL-8.
- Detection Range: 31.2 – 2000 pg/ml
- Sensitivity: 18.75 pg/ml
- Intra-Assay: CV<8%
- Inter-Assay: CV<10%
- This assay has high sensitivity and excellent specificity for the detection of IL-8. No significant cross-reactivity or interference between IL-8 and analogues was observed.

III. Specificity:

Human

IV. Sample Types:

Human serum, plasma, tissue homogenates and other biological fluids.

V. Kit Contents:

Components	K4169-100	Part No.
Micro ELISA Plate	8 x 12 strips	K4169-100-1
Lyophilized Standard	2 vials	K4169-100-2
Sample / Standard dilution buffer	20 ml	K4169-100-3
Biotin- detection antibody (Concentrated)	120 µl	K4169-100-4
Antibody dilution buffer	10 ml	K4169-100-5
HRP-Streptavidin Conjugate (SABC) (Avoid light)	120 µl	K4169-100-6
SABC dilution buffer	10 ml	K4169-100-7
TMB substrate (Avoid light)	10 ml	K4169-100-8
Stop Solution	10 ml	K4169-100-9
Wash buffer (25X)	30 ml	K4169-100-10
Plate sealers	5	K4169-100-11

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37 °C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

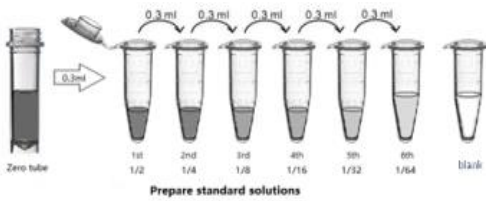
VII. Storage and Handling:

The entire kit may be stored at 4 °C for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare the reagents within 30 min before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Biotin- detection antibody working solution:** Calculate the total volume of the working solution: 0.1 ml / well x quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.
2. **HRP-Streptavidin Conjugate (SABC):** Calculate the required total volume of the working solution: 0.1 ml / well x quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.
3. **Wash Buffer:** Dilute 30 ml of Concentrated Wash Buffer (25X) into 750 ml of Wash Buffer with deionized or distilled water. Put unused solution back at 4 °C. If crystals have formed in the concentrate, warm it with 40 °C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature (RT) before use.
4. **Standard Preparation:**
   - Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at RT for 10 min and mix them thoroughly. **Note:** If the Standard tube concentration higher than the range of the kit, dilute it and label as zero tube.
   - Label 7 tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml of the Sample Dilution Buffer into each tube. Add 0.3 ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer is used for the blank control. Allow solution to sit at RT for 10 min and gently vortex to mix completely. **Note:** It is best to use Standard



Prepare standard solutions

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Solutions within 2 hr.

- Suggested Standard points are: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/ml.

5. Sample Preparation:

Note: Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20 °C (≤1 month) or -80 °C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

- **Serum:** Place whole blood sample at RT for 2 hr or put it at 2-8 °C overnight and centrifuge for 20 min at approx 1000 x g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- **Plasma:** Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000 x g at 2-8 °C within 30 min of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** As hemolysis blood has relation to assay result, it is necessary to remove the residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS with a glass homogenizer on ice. The volume depends on the weight of the tissue. Some protease inhibitors are recommended to add into the PBS with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000 x g to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 gm.
- **Cell culture supernatant:** Centrifuge the supernatant for 20 min at 1000 x g at 2-8 °C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
- **Cell Culture Lysate:** Commercial RIPA kits are recommended to follow the instructions provided. Generally, 0.5 ml RIPA lysis buffer would be appropriate to 2 x 10^6 cells, DNA must to be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
- **Other biological fluids:** Centrifuge samples for 20 min at 1000 x g at 4 °C. Collect the supernatant and carry out the assay immediately.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

IX. Assay Protocol:

Note: Bring all reagents and samples to RT, 30 min prior to the assay. It is recommended that all Standards and samples be run at least in duplicates. A Standard Curve must be run with each assay. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C. It is recommended to plot a Standard Curve for each test.

1. Prepare all reagents, samples and standards as instructed above.
2. Wash plate 2 times with **1X Wash Solution** before adding standard, sample and control wells.
3. Add 100 µl of each **standard** and **samples** into appropriate wells and 100 µl of Sample Dilution Buffer into the **blank** wells. Seal the plate with a cover and incubate for 90 min at 37 °C.
4. Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
5. Add 0.1 ml of **Biotin-detection antibody** working solution into the above wells including Standard, test sample and blank wells. Seal the plate and incubate at 37 °C for 60 min.
6. Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 min each time. Then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
7. Add 0.1 ml of **SABC working solution** into each well, cover the plate and incubate at 37 °C for 30 min.
8. Discard the solution and wash 5 times with **1X Wash Solution** as step 6.
9. Add 90 µl of **TMB Substrate** into each well, cover the plate and incubate at 37 °C in dark within 10-20 min. The shades of blue should be seen in the first 3-4 wells by the end of incubation. **Note:** The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. The end user can terminate the reaction when apparent gradient appeared in standard wells.
10. Add 50 µl of **Stop Solution** to each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
11. Read the O.D. at 450nm in Microplate Reader immediately after adding the stop solution.

X. Calculation:

For calculation, **(the relative O.D$_{450\ nm}$) = (the O.D $_{450\ nm}$ of each well) – (the O.D$_{450\ nm}$ of Zero well).** The Standard Curve can be plotted as the relative O.D$_{450\ nm}$ of each standard solution (Y) vs. the respective concentration of the Standard solution (X). The Human IL-8 concentration of the samples can be interpolated from the Standard Curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.

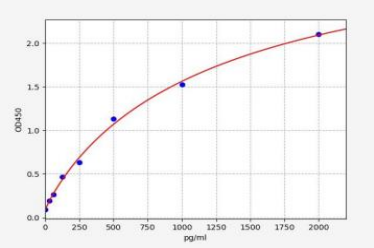

Figure: Typical Standard Curve. The Standard Curve are for demonstration only. A Standard Curve must be run with each assay.

XI. Related Products:

IL-8 Antibody (Cat. No. 5149-100)
IL-10 (Human) ELISA Kit (Cat. No. K4167-100)
IL-4 (Human) ELISA Kit (Cat. No. K4164-100)
IL-8 (77 a.a.), human recombinant (Cat. No. 4149-25, -1000)
IL-5 (Human) ELISA Kit (Cat. No. K4156-100)

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