Human IL-8/CXCL8 ELISA Kit

Cat No. ELI-M-012-96
Size: 96 tests

MOLEQULE-ON®

Components	Quantity
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 Plate
Standard - lyophilized, 600 pg/ml upon reconstitution	3 Vials
Concentrated Biotin-Conjugated antibody	1 Vial
Concentrated Streptavidin-HRP solution	1 Vial
Reagent Diluent – 21ml/vial	1 Bottle
Assay Buffer Concentrate (20X) – 20ml/vial	1 Bottle
20X PBS Buffer – 20ml/vial	1 Bottle
Substrate Solution – 10.5 ml/vial	1 Bottle
Stop Solution – 5.5 ml/vial	1 Bottle
Plate Cover Seals	2 pieces

Reagent Preparations

1. Temperature returning - Bring all kit components and specimen to room temperature (20-25℃) before use.

2. Working Solution of Assay Buffer: Dilute 20ml of Assay Buffer Concentrate with 380ml of deionized or distilled water to prepare 400ml of 1X Assay Buffer. If crystals have formed in the concentrate Assay Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. Working Solution of PBS: Dilute 20ml of 20X PBS with 380ml of deionized or distilled water to prepare 400ml of 1X PBS.

3. Standard/Specimen - Reconstitute the Standard with 500µl of 1X Assay Buffer. This reconstitution produces a stock solution of 600pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µl of 1X Assay Buffer into six tubes and marked them as 300pg/ml, 150pg/ml, 75pg/ml, 37.5pg/ml, 18.75pg/ml and 9.38pg/ml. Make a serial dilution by taking 250µl standard from stock (600pg/ml) and dispense into next dilution (300pg/ml) then pipette 250µl from first dilution (300pg/ml) to the next tube (150pg/ml) and subsequently add 250µl from each standard tube to next tube to make serial dilution of mentioned concentration. Mix each tube thoroughly and change pipette tips between each transfer. The 600pg/ml standard serves as the high standard. The 1X Assay Buffer serves as the zero standard (0pg/ml). Serially diluted standard shall not be reused.

4. Working solution of Biotin-Conjugate anti-human IL-8/CXCL8 antibody: Centrifuge for 1 min at 6000 rpm to bring down the material prior to open the vial. Add 200ul of sterile 1X PBS to the vial and vortex the reconstituted antibody for 30 seconds. Add 20µl of reconstituted Biotin-conjugate solution to 980µl of Reagent Diluent to prepare working solution of Biotin-Conjugate anti-human IL-8/CXCL8 antibody. If the entire 96-well plate is used, take the entire 200ul of antibody to 10ml of Reagent Diluent and mix thoroughly prior to the assay. If the partial antibody is used store the rest at -20°C until use.
***The working solution should be used within one day after dilution.**

5. Working solution of Streptavidin-HRP: Centrifuge for 1 min at 6000 rpm to bring down the material prior to open the vial. The vial contains 50µL HRP Conjugate sufficient for one 96-well plate. (If the volume is less than 50µL, add sterile 1X PBS to reach 50µL and vortex for 10 seconds). Add 5µl of Streptavidin-HRP Conjugate to 995µl of Reagent Diluent to prepare working solution of streptavidin-HRP. If the entire 96-well plate is used, add 50□L of HRP Conjugate to 10ml of Reagent Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.
***The working solution should be used within one day after dilution.**

Storage

All components of Human IL-8/CXCL8 ELISA Kit should be store at 2-8°C except reconstituted standard that should be store at -20℃.

Description

IL-8 was renamed CXCL8 by the Chemokine Nomenclature Subcommittee of the International Union of Immunological Societies, although its approved HUGO gene symbol remains IL8. CXCL8 was was also referred to as neutrophil chemotactic factor (NCF), neutrophil activating protein (NAP), monocytederived neutrophil chemotactic factor (MDNCF), Tlymphocyte chemotactic factor (TCF), granulocyte chemotactic protein (GCP) and leukocyte adhesion inhibitor (LAI). Many cell types, including monocyte/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, keratinocytes, hepatocytes, chondrocytes, and various tumor cell lines, can produce CXCL8 in response to a wide variety of proinflammatory stimuli such as exposure to IL1, TNF, LPS, and viruses. CXCL8 is a potent chemoattractant for neutrophils.

Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-8/CXCL8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8/CXCL8 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for IL-8/CXCL8 is added to detect the captured IL-8/CXCL8 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by Tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity, which is proportional to the quantity of bound protein is measurable at 450nm.

Sample Collection And Storage

1. Cell Culture Supernates - Centrifuge cell culture media at 1000 x g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

2. Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000g (or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

3. Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g (or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

Procedure

Prepare all reagents and working standards as directed in the previous section.

1. Add 100□L of sample (such as plasma or serum) or standard per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1X Assay Buffer. Wash by filling each well with 300µl of 1X Assay Buffer using a multi-channel pipette, semi-automated or automated washer. Complete removal of liquid at each step is essential for good performance. **Repeat the washing step 4 times**. After the last wash, remove any remaining Assay Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100□L of the working dilution of Biotin-Conjugate anti-human IL-8/CXCL8 antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100□L of the working dilution of HRP Conjugate to each well. Cover the plate and incubate for 25 minutes at room temperature. Keep the plate in dark condition.
6. Repeat the aspiration/wash as in step 2.
7. Add 100□L of Substrate Solution to each well. Incubate for 5-20 minutes (depending on signal) at room temperature. Keep the plate in dark condition.
8. Add 50□L of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Technical Note

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

Calculation of Results

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the IL-8/CXCL8 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Sensitivity, Specificity And Repeatability

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay was less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 2pg/ml.
3. **SPECIFICITY**: This assay recognizes both natural and recombinant human IL-8/CXCL8.

References

1. Bacon K, et al (October 2002). J. Interferon Cytokine Res. 22 (10): 1067–8.
2. Vlahopoulos S, et al. (September 1999). Blood 94 (6): 1878–89.
3. Utgaard JO, et al (November 1998). J. Exp. Med. 188 (9): 1751–6.