Human IL-8 (CXCL8) ELISA Kit

For detection and measurement of human interleukin-8 (CXCL8)

Catalog #100-1143 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
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Product Description

The Human Interleukin-8 (IL-8) CXCL8 ELISA Kit is designed for the quantitative detection and measurement of human IL-8, also known as CXCL8, in cell culture supernatants. IL-8 is secreted by monocytes, macrophages, T cells, neutrophils, fibroblasts, and endothelial cells in response to proinflammatory stimuli such as IL-1 and TNF-α. IL-8 mediates its functions by binding to CXCR1, CXCR2, and DARC receptors, which produce different biological effects. CXCR1 and CXCR2 are G protein-coupled receptors mainly expressed on leukocytes, endothelial cells, epithelial cells, and neuronal cells. The DARC receptor acts as a silent receptor, clearing ELR+ angiogenic chemokines and inhibiting tumor development. The production of IL-8 in biological fluids can be induced by bacteria (e.g. *Helicobacter pylori*), lipopolysaccharides, viruses (e.g. respiratory syncytial, rhinovirus), and viral products (e.g. X protein of human hepatitis virus B).

The assay is based on a sandwich ELISA method, in which samples are added to ELISA strip plates pre-coated with capture antibodies specific for human IL-8.The captured IL-8 is detected by the addition of a horseradish peroxidase (HRP)-conjugated detection antibody. The addition of the chromogenic enzyme substrate 3,3'5,5' tetramethylbenzidine (TMB) results in a colored product with an intensity directly proportional to the concentration of human IL-8 in the sample.

Product Information

All components listed below are stable until expiry date (EXP) on label. Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated upon receipt.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE AND STABILITY	DESCRIPTION
Human IL-8 ELISA Plate	300-0772	1 plate	Store at 2 - 8°C. Use within 1 month of opening.	Plate (12 strips x 8 wells) coated with anti-human IL-8 antibody
Human IL-8 HRP-Conjugated Detection Antibody	300-0773	1 vial	Store at 2 - 8°C. Use within 1 month of opening.	HRP-conjugated anti-human IL-8 antibody
Human IL-8 Standard	300-0774	1 vial	Store at 2 - 8°C. Use within 1 month of opening.	Lyophilized recombinant human IL-8
Wash Buffer (20X)*	300-0775	30 mL	Store at 2 - 8°C. Use within 1 week of opening.	Concentrated buffer solution for washing plates
Dilution Buffer (20X)*	300-0776	12.5 mL	Store at 2 - 8°C. Use within 1 week of opening.	Protein-containing buffer for dilution of samples, standard, and detection antibody
Standard Dilution Buffer	300-0777	2 mL	Store at 2 - 8°C. Use within 1 week of opening.	For reconstitution of lyophilized cytokine standard
Peroxide Solution	300-0778	12.5 mL	Store at 2 - 8°C. Use within 1 month of opening.	Stabilized hydrogen peroxide
TMB Substrate	300-0779	12.5 mL	Store at 2 - 8°C. Use within 1 month of opening.	3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution containing hydrogen peroxide
Stop Solution	300-0780	11 mL	Store at 2 - 8°C. Use within 1 month of opening.	0.25 M hydrochloric acid

* Please refer to the Safety Data Sheet (SDS) relevant to your region for hazard information.

Materials Required but Not Included

- Biohazard safety cabinet certified for Level II handling of biological materials
- Microplate reader set at a wavelength of 450 nm
- 37°C incubator
- ELISA plate washer: Automatic (adaptable for ELISA strip plates) or manual (e.g. multi-pipette or squirt bottle)
- Micropipette (e.g. Eppendorf, Gilson) with appropriate tips

- Beakers, flasks, and graduated cylinders necessary for reagent preparations
- Tubes for standard and sample dilutions
- Timer
- Absorbent paper
- Distilled or deionized water
- Well plate cover or seals

Preparation of Reagents and Materials

A. Wash Buffer

NOTE: Use clean or disposable glass or plasticware for preparation and storage of wash buffer.

NOTE: If crystals are visible in the concentrate, warm to room temperature (15 - 25°C) and mix gently until completely dissolved.

Dilute Wash Buffer (20X) 1 in 20 with distilled or deionized water. Use on day of preparation; do not store.

Example: Prepare 400 mL wash buffer by adding 20 mL Wash Buffer (20X) to 380 mL of distilled or deionized water.

B. Dilution Buffer

NOTE: Use clean or disposable glass or plasticware for preparation and storage of wash buffer.

NOTE: If crystals are visible in the concentrate, warm to room temperature (15 - 25°C) and mix gently until completely dissolved.

Dilute Dilution Buffer (20X) 1 in 20 with distilled or deionized water. Use on day of preparation; do not store.

Example: Prepare 100 mL of dilution buffer by adding 5 mL of Dilution Buffer (20X) to 95 mL of distilled or deionized water.

C. ELISA Plates

1. Allow plates to adjust to room temperature (15 - 25°C) before opening the bags.
2. Plan the experiment to include a standard curve and a background control (8 x 2 wells), a blank (2 wells), and sample wells.
3. Assemble the required number of strips in the plate frame. Store the remaining strips in the vacuum bag at 2 - 8°C.
4. Wash the strips with 2 x 300 µL/well of wash buffer (prepared in section A). Ensure wash buffer is thoroughly removed from the wells by firmly tapping the plate upside down on absorbent paper.

D. Standard Stock Solution

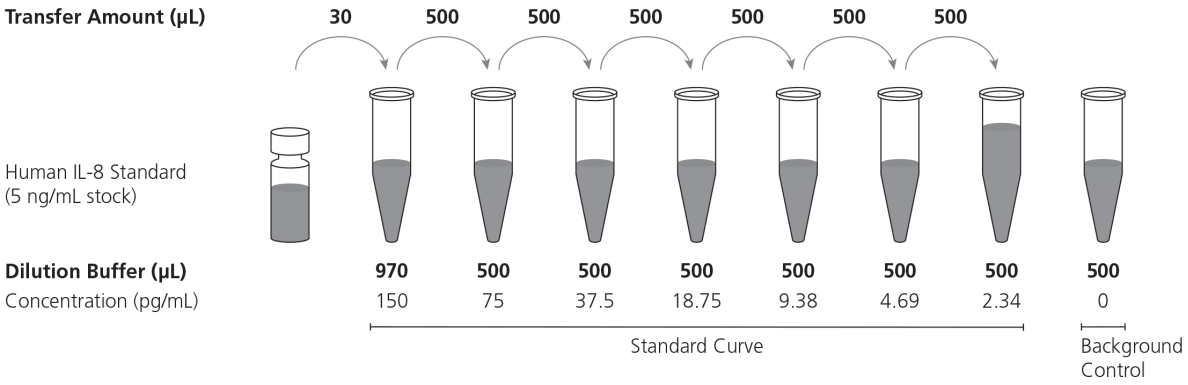
1. Centrifuge the standard vial to bring all material to the bottom of the vial.
2. Reconstitute Human IL-8 Standard with Standard Dilution Buffer to a final concentration of 5 ng/mL. The mass of the standard is provided on the vial label.
3. Mix thoroughly until fully dissolved (do not invert vial). Use immediately; do not store.

E. Standard Curve

NOTE: The standard curve dilutions may be prepared from freshly prepared standard stock solution or from thawed aliquots. Prepare the standard curve dilutions no more than 30 minutes prior to beginning the assay. Duplicate wells for the standard curve dilutions and the background control are recommended.

Dilute the standard stock solution (prepared in section D) to create standard curve dilutions ranging from 2.34 - 150 pg/mL according to Figure 1.

For the background control (0 pg/mL), use dilution buffer only.



NOTE: Volumes indicated are sufficient for duplicates.

FIGURE 1. Recommended Serial Dilution of Cytokine Standard

F. Detection Antibody

Dilute Human IL-8 HRP-Conjugated Detection Antibody with Standard Dilution Buffer within 10 minutes of use. For the lot-specific dilution factor, refer to the Certificate of Analysis (CoA) available at www.stemcell.com/coa.

G. Substrate Solution

To prepare substrate solution, combine equal volumes of TMB Substrate and Peroxide Solutions and mix. Use within 10 minutes of preparation. Protect from light.

NOTE: Do not use if the Substrate Solution appears blue after preparation.

Directions for Use

Please read the entire protocol before proceeding. In all washing steps, each well must be thoroughly washed and any remaining wash buffer removed by aspirating or decanting, then blotting dry by tapping the plate upside down on absorbent paper. Duplicate wells for all samples and standards are recommended.

NOTE: Cross-contamination of reagents may invalidate assay results. Permanently labeled, dedicated, multi-channel micropipette reservoirs for reagents are recommended.

1. Bring all reagents and samples to room temperature (15 - 25°C).
2. Prepare all buffers, reagents, samples, standard dilutions, and ELISA plate as described in Preparation of Reagents and Materials.
3. Add 100 µL/well of each standard curve dilution and background control (see Preparation of Reagents and Materials, section E). Leave the blank wells empty.
4. Add 100 µL/well of diluted sample.

   NOTE: Duplicate wells for all samples are recommended.
5. Cover or seal the plate and incubate at room temperature for 2 hours.
6. Empty wells. Wash each well with 3 x 300 µL of wash buffer (see Preparation of Reagents and Materials, section A) and blot dry.
7. Add 100 µL/well of diluted detection antibody (see Preparation of Reagents and Materials, section F). Leave blank wells empty.
8. Cover or seal the plate and incubate at room temperature for 1 hour.
9. Empty wells. Wash each well with 3 x 300 µL of wash buffer and blot dry.
10. Add 100 µL/well of substrate solution (see Preparation of Reagents and Materials, section G). Leave blank wells empty.
11. Cover or seal the plate and incubate at room temperature for 20 minutes.
12. Add 100 µL/well of Stop Solution to all wells (including blank wells) in the same order as substrate solution. Gently tap the plate to ensure thorough mixing.

    NOTE: Use care when handling the Stop Solution. Refer to the Safety Data Sheet (SDS) for hazard information.
13. Within 10 minutes of adding Stop Solution, measure the absorbance at 450 nm in a microplate reader. Blank the reader using ELISA wells containing substrate solution and Stop Solution only.

    NOTE: Before measuring absorbance, remove any air bubbles in the wells using a small hypodermic needle or a pipette tip.
14. Calculate the average absorbance of each sample from the duplicate values. Subtract the mean absorbance value of the blank from the standard, the background control, and the sample values prior to generating the standard curve and determining the cytokine concentrations in the samples. A representative standard curve is shown in Figure 2.

    NOTE: Multiply the cytokine concentrations by the dilution factor used for each sample.

Performance of the Assay

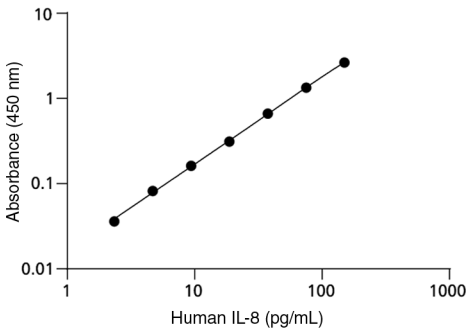


FIGURE 2. Representative Standard Curve

- Reportable range: 2.34 - 150 pg/mL. This is the concentration range in which measurement of the analyte can be done with the highest precision, accuracy, and linearity.
- Sensitivity: The limit of detection of this assay is 0.75 pg/mL. This is the analyte concentration with absorbance two standard deviations higher than the zero standard.
- Recovery: A mid-curve recovery of 72 - 109% was determined by spiking defined amounts of analyte standard into cell culture supernatant samples in repeated experiments.
- Precision: The intra-assay precision of this assay is 4.2% (CV). The inter-assay precision of this assay is 1.1% (CV).

Troubleshooting

PROBLEM	POSSIBLE CAUSE	RECOMMENDED ACTION
Absorbance values too low	Incubation time too short	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Undiluted Wash Buffer (20X)	Dilute Wash Buffer (20X) with distilled or deionized water as described in Preparation of Reagents and Materials, section A.
	Incorrect dilution of Detection Antibody and/or preparation of substrate solution	Follow the directions for dilution of Detection Antibody and preparation of substrate solution in Preparation of Reagents and Materials.
Absorbance values too high	Incubation time too long	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Temperature too high (> 25°C)	Perform assay at a lower temperature. If that is not possible, reduce incubation times.
	Incorrect dilution of Detection Antibody and/or preparation of substrate solution	Follow the directions for dilution of Detection Antibody and preparation of substrate solution in Preparation of Reagents and Materials.
Low absorbance readings despite good color development in wells	Incorrect wavelength setting on the ELISA reader	Check that the measuring wavelength is set at 450 nm and that the ELISA reader has the correct filter for this wavelength.
High background	Insufficient washing	Ensure that each well is washed 3X with 300 µL of diluted Wash Buffer (20X). Ensure that multi-channel pipette fills and empties reproducibly without touching the reagents on the plate. Blot wells dry before proceeding to the next step. Increase cycles of washes and soaking time between washes.
	Substrate solution was not clear and colorless prior to addition	Do not use the substrate solution if it appears blue after preparation.
	Incorrect dilution of Detection Antibody and/or preparation of substrate solution	Follow the directions for dilution of Detection Antibody and preparation of substrate solution in Preparation of Reagents and Materials.
No signal with standard curve dilutions	Reagents not added in correct sequence	Follow sequence of incubation and wash steps as outlined in Directions for Use.
	Incorrect storage condition	Check if the kit is stored at the recommended storage condition and used before expiration date.

PROBLEM	POSSIBLE CAUSE	RECOMMENDED ACTION
	Incorrect dilution of Detection Antibody and/or preparation of substrate solution	Follow the directions for dilution of Detection Antibody and preparation of substrate solution in Preparation of Reagents and Materials.
Poor standard curve	Incubation conditions for individual wells not identical	Avoid delays during filling of assay wells; ensure that all reagents are at 15 - 25°C prior to beginning the assay.
	Imprecise or inaccurate pipetting	Ensure that pipettes function properly.
	Incubations performed at inappropriate temperature, timing, or agitation	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Background wells were contaminated	Avoid cross-contamination by using the sealer appropriately.
Poor replicates	Insufficient washing	Ensure that each well is washed 3X with 300 µL of diluted Wash Buffer (20X). Ensure that multi-channel pipette fills and empties reproducibly without touching the reagents on the plate. Blot wells dry before proceeding to the next step. Increase cycles of washes and soaking time between washes.
	Unequal volumes in wells	Ensure that pipettes function properly.
	Carry-over between wells; evaporation from wells; splashing of well contents onto adhesive cover	Always use a new adhesive cover for each incubation. Ensure that each well is sealed tightly. Keep filled plates in a horizontal position and handle with caution.
	Samples not mixed after thawing	Vortex samples after thawing.
	High lipids or particulate matter in samples	Filter or centrifuge samples to pellet aggregates. Lipids may concentrate on the surface after centrifugation and may be removed.
	Air bubbles in well during measurement with the ELISA reader	Remove air bubbles using a small hypodermic needle or pipette tip.
Absorbance values of sample dilutions decrease with increasing concentration	Cytokine concentration of the sample dilutions exceeds the upper limit of the assay	Dilute the samples further and retest along with the standard curve dilutions.

Related Products

For a complete list of ELISA kits and related products from STEMCELL Technologies, visit www.stemcell.com, or contact us at techsupport@stemcell.com.