ab48481 –
Human IL-8 ELISA Set (without plates)

Instructions for Use

For the quantitative measurement of Human IL-8 in cell culture supernatants, buffered solutions, serum, plasma and other body fluids.

This product is for research use only and is not intended for diagnostic use.

Version 1f Last Updated 9 February 2022

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1. BACKGROUND

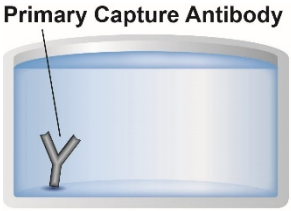
Abcam's Human IL-8 *in vitro* ELISA Set is designed for the quantitative measurement of IL-8 in cell culture supernatants, buffered solutions, serum, plasma and other body fluids.

An antibody specific for IL-8 is coated onto the wells of the microtiter plates. Samples, including standards of known IL-8 concentrations and unknowns are pipetted into these wells and incubated at room temperature. The wells are then washed and a biotinylated antibody specific for IL-8 is added to the wells and incubated. After further washing, a Streptavidin-peroxydase conjugate is added to each well, and incubated. The wells are then washed to remove all unbound enzyme and TMB solution, which acts on the bound enzyme is added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IL-8 present in the samples.

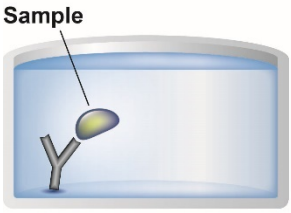
Interleukin 8 (IL-8) or CXCL8, Monocyte-Derived Neutrophil Chemotactic Factor (MDNCF), Neutrophil Activating Factor (NAF) and NAD-P1 is a chemokine secreted by monocytes, macrophages and endothelial cells. IL-8 chemoattracts and activates neutrophils. The predominant form of IL-8 is a 8.4kDa protein containing 72 amino acid residues, which includes five additional N-Terminal amino-acids. IL-8 contains the four conserved cysteine residues present in CXC chemokines and also contains the "ELR" motif common to CXC chemokines that binds to CXCR1 and CXCR2. IL-8 may participate in the pathogenesis of rheumatoid arthritis via the induction of neutrophil-mediated cartilage damage, and psoriasis. A causative involvement of IL-8 is found within a broad range of clinico-pathological conditions: adult respiratory distress syndrome, asthma, bacterial infections, bladder cancer, graft rejection and influenza infection, due to the now known biological properties of IL-8. This cytokine, especially in combinations with other neutrophil activating agents, may prove helpful in the treatment of patients suffering from granulocytopenia,

severe infections against which antibiotics are not effective, and immunodeficiency caused by HIV. This kit will recognise both endogenous and recombinant Human IL-8.

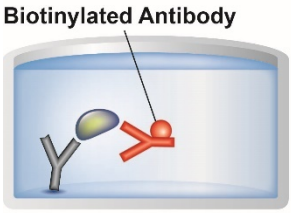
2. ASSAY SUMMARY



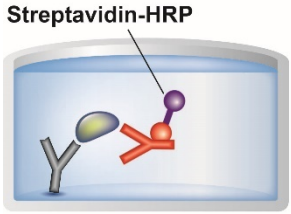
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



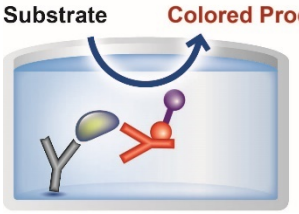
Add standard or sample to each well used.



Add biotinylated labeled detector antibody to each well used. Incubate at room temperature.



Aspirate and wash each well. Add prepared Streptavidin-HRP mix to each well. Incubate at room temperature.



Aspirate and wash each well. Add the TMB Solution to each well until color develops and then add the Stop Solution. Immediately begin recording the color development.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Quantity			Storage Condition (Before Preparation)
	5 x 96 tests	10 x 96 tests	15 x 96 tests	
IL-8 Capture antibody	1 x 100 µL	2 x 100 µL	3 x 100 µL	+2-8ºC
Detection biotinylated anti IL-8 antibody	1 x 1 vial	2 x 1 vial	3 x 1 vial	+2-8ºC
IL-8 standard	5 x 1 vial	10 x 1 vial	15 x 1 vial	+2-8ºC
Streptavidin HRP	1 x 1 vial	2 x 1 vial	3 x 1 vial	+2-8ºC
Ready-to-Use TMB	2 x 1 vial	4 x 1 vial	6 x 1 vial	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96 well Microtitre plates.
- Reconstitution Buffer

  1X PBS + 0.09% Azide. Once prepared store at 2-8°C for up to one week.
- Coating Buffer

  1X PBS, pH 7.2-7.4. Once prepared store at 2-8°C for up to one week.
- Wash Buffer

  1X PBS + 0.05% Tween20. Once prepared use immediately.
- Blocking Buffer

  1X PBS + 5% BSA. Once prepared store at 2-8°C for up to one week.
- Standard and Secondary Antibody Dilution Buffer

  1X PBS + 1% BSA. Once prepared store at 2-8°C for up to one week.
- HRP Diluent Buffer

  1X PBS + 1% BSA + 0.1% Tween20. Once prepared store at 2-8°C for up to one week.
- Stop Reagent (1M Sulfuric Acid).
- Microtitre plate reader with appropriate filters (450 nm required with optional 620 nm reference filter).
- Microplate washer or wash bottle.
- 10, 50, 100, 200 and 1,000 µL adjustable single channel micropipettes with disposable tips.
- 50-300 µL multi-channel micropipette with disposable tips.
- Multichannel micropipette reagent reservoirs.
- Distilled water.

- Vortex mixer.
- Miscellaneous laboratory plastic and/or glass, if possible sterile.

7. LIMITATIONS

- Exact conditions may vary from assay to assay, a standard curve should be generated for every assay performed.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh 1X Wash Buffer. Do not allow wells to sit uncovered or dry for extended periods.
- Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

8. TECHNICAL HINTS

- Kit components should be stored as indicated. All the reagents should be equilibrated to room temperature before use. Reconstituted standards should be discarded after use.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination; for the dispensing of the Stop Solution and substrate solution, avoid pipettes with metal parts.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly.
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.
- Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Dispense the TMB solution within 15 minutes following the washing of the microtiter plate.

This kit is sold based on number of tests. A 'test' simply refers to a single assay well. Please contact our Technical Support staff with any questions.

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 **Capture Antibody**

For 1 x 96 well plates add 20 µL of Capture Antibody into 10 mL of Coating Buffer.

9.2 **Reconstituted Biotinylated anti IL-8 Detection Antibody**

It is recommended this reagent is prepared **immediately before use**. Reconstitute each vial with 550 µL PBS + 0.1% Azide w/v or another preservative. Dilute the reconstituted biotinylated anti-IL-8 with the Standard and Secondary Antibody Dilution Buffer in an appropriate clean glass vial. For 1 x 96 well plates add 100 µL of the reconstituted Detection Antibody into 5 mL of Standard and Secondary Antibody Dilution Buffer. Once prepared store at 2-8°C for up to one year.

9.3 **Preparation of Streptavidin-HRP**

It is recommended to centrifuge the vial for a few seconds in a microcentrifuge to collect all the volume at the bottom of the vial. Dilute 5 µL of Streptavidin-HRP into 500 µL of HRP Diluent Buffer **immediately before use.** For 1 x 96 well plates take 150 µL of the diluted HRP solution into 10 mL of HRP Diluent Buffer. Do-not keep these solutions for future experiments.

10. STANDARD PREPARATION

- Standard vials must be reconstituted with the volume of standard dilution buffer shown on the vial immediately prior to use.

10.1. Reconstitute the IL-8 standard sample by adding the volume of Standard Dilution Buffer indicated on the vial label by pipette. Mix thoroughly and gently. This is the 1,000 pg/mL **Standard #1** Solution (see table below).

Note: The reconstituted Standard #1 should be discarded after use and not stored for reuse.

10.2. Label six tubes with Standards # 2 – 7.

10.3. Add 300 µL Standard Dilution Buffer into Standards # 2 - 6 and 500 µL Standard Dilution Buffer into Standard #7.

10.4. Prepare Standard #2 by transferring 300 µL from Standard #1 to Standard #2. Mix thoroughly and gently.

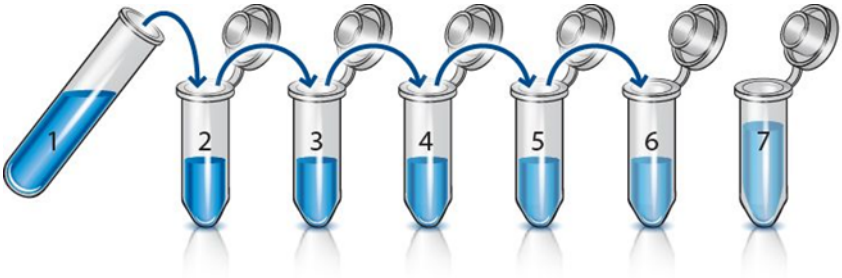
10.5. Prepare Standard #3 by transferring 300 µL from Standard #2 to Standard #3. Mix thoroughly and gently.

10.6. Using the table below as a guide, repeat for Standards #4 through to Standard #6.

10.7. Standard #7 contains no protein and is the Blank control.

Standard Dilution Preparation Table

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	See Step 10.1				1,000
2	Standard #1	300	300	1,000	500
3	Standard #2	300	300	500	250
4	Standard #3	300	300	250	125
5	Standard #4	300	300	125	62.5
6	Standard #5	300	300	62.5	31.25
7 (Blank)	-		500	0	0



11. SAMPLE PREPARATION AND STORAGE

- Cell culture supernatants, Human serum, plasma or other biological samples will be suitable for use in the assay.
- If not analyzed shortly after collection, samples should be aliquoted (250-500 µL) to avoid repeated freeze-thaw cycles and stored frozen at -70°C. Avoid multiple freeze-thaw cycles of frozen specimens.
- Do not thaw samples by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use. When possible avoid use of badly haemolysed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

11.1. Cell culture supernatants

Remove particulates and aggregates by centrifuging at approximately 1,000 x g for 10 minutes.

11.2. Serum

Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following clotting, centrifuge at approximately 1,000 x g for 10 minutes and remove serum.

11.3. Plasma

EDTA, citrate and heparin plasma can be assayed. Centrifuge samples at 1,000 x g for 30 minutes to remove particulates. Harvest plasma.

12. PLATE PREPARATION

- If you wish to store the coated and blocked plates for future use, bench dry each plate at room temperature (18 to 25°C) for 24 hours. Cover the plates and then store at 2-8°C in a sealed plastic bag with desiccant for up to 12 months.

12.1. Add 100 µL of diluted Capture Antibody to each well. Cover with a plastic plate cover and incubate at 4°C overnight.

12.2. Remove the cover and wash the plate as follows:

12.2.1. Aspirate the liquid from each well.

12.2.2. Dispense 400 µL of washing solution into each well.

12.2.3. Aspirate the contents of each well.

12.2.4. Repeat step 12.2.2 and 12.2.3.

12.3. Add 250 µL of Blocking Buffer to each well.

12.4. Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 2 hours.

12.5. Remove the cover and wash the plate as follows:

12.5.1. Aspirate the liquid from each well.

12.5.2. Dispense 400 µL of washing solution into each well.

12.5.3. Aspirate the contents of each well.

12.5.4. Repeat step 12.5.2 and 12.5.3 two times.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **We strongly recommend that every vial is mixed thoroughly without foaming prior to use except the standard vial which must be mixed gently by inversion only.**
- **Note: Final preparation of Biotinylated anti-IL-8 and Streptavidin-HRP should occur immediately before use.**

13.1 Prepare Standard curve as shown in Section 10.

13.2 Add 100 µL of each standard or sample to appropriate wells in duplicate.

13.3 Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 1 hour.

13.4 Remove the cover and wash the plate as follows:

13.4.1 Aspirate the liquid from each well

13.4.2 Dispense 300 µL of washing solution into each well

13.4.3 Aspirate the contents of each well

13.4.4 Repeat step 13.4.2 and 13.4.3.

13.5 Add 50 µL of diluted Detection Antibody into all wells.

13.6 Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 30 minutes.

13.7 Repeat wash step 13.4.

13.8 Add 100 µL of Streptavidin-HRP solution into all wells.

13.9 Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 30 minutes.

13.10 Repeat wash step 13.4.

13.11 Add 100 µL of ready-to-use TMB Substrate Solution into all wells.

13.12 Incubate in the dark for 5-15 minutes* at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.

**Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*

13.13 Add 100 µL of Stop Reagent into all wells.

13.14 Read the absorbance value of each well (immediately after step 13.13.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).

14. CALCULATIONS

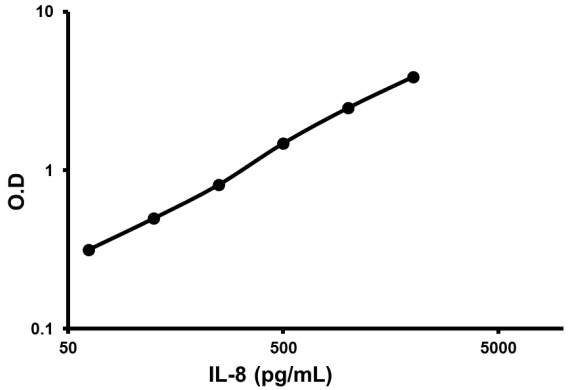
Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding IL-8 standard concentration on the horizontal axis.

The amount of IL-8 in each sample is determined by extrapolating OD values against IL-8 standard concentrations using the standard curve.

15. TYPICAL DATA

TYPICAL STANDARD CURVE - Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D.
0	0.122
62.5	0.316
125	0.497
250	0.809
500	1.481
1000	2.477
2000	3.887

Figure 1. Example of typical IL-8 standard curve data.

16. ASSAY SPECIFICITY

The assay recognizes natural Human IL-8. To define specificity of this IL-8 antibody pair, several proteins were tested for cross reactivity using the Abcam IL-8 pre-coated ELISA kit (which contains the same antibodies).There was no cross reactivity observed for any protein tested (IL-1α, IL-1β, IL-10 IL-12, IFNγ, IL-2, IL-4, IL-6, TNFα and IL-13).

17. ASSAY SENSITIVITY

The sensitivity, minimum detectable dose of this IL-8 antibody pair was determined using the Abcam IL-8 ELISA kit (which contains the same antibodies) and was found to be <12.3 pg/mL. This was determined by adding 3 standard deviations to the mean OD obtained when the zero standard was assayed 40 times.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components +2-8°C. Keep substrate solution protected from light.

19. NOTES

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)

All information / detail is correct at time of going to print.