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ELISA Flex: Monkey IL-8 (ALP)

3560M-1A-6 | 3560M-1A-20

ELISA Flex kit for quantitative determination of native monkey IL-8 (CXCL8) in solution, e.g. cell supernatant.

The kit includes		3560M-1A-6 for 6 plates	3560M-1A-20 for 20 plates
Capture mAb:	MT8H6 (0.5 mg/ml)	300 µl	1000 µl
Detection mAb:	26E5, biotin (0.5 mg/ml)	50 µl	150 µl
Streptavidin-ALP		80 µl	250 µl
Recombinant human IL-8 (CXCL8) ELISA standard		1 vial	1 vial
Standard reconstitution buffer A5		1 ml	1 ml

To ensure total recovery of the stated quantity, vials have been overfilled.

Shipping and storage

Shipped at ambient temperature. All reagents should be stored at 4-8 °C upon receipt, except the standard which should be stored at -20 °C. Antibodies are supplied in sterile-filtered PBS with sodium azide (0.02%). Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

General and Preparations

Specificity

The kit contains a matched pair of monoclonal antibodies (mAbs) specific for native and recombinant human IL-8 (CXCL8). The mAbs cross-react with IL-8 from non-human primates. Please visit www.mabtech.com for reactivity on NHP species. The mAbs also cross-react with IL-8 from cow, thus the use of bovine serum in cell-cultures is not recommended.

Standard range

8-800 pg/ml

Calibration

No international standard exists for calibration.

Analysis of serum and plasma samples

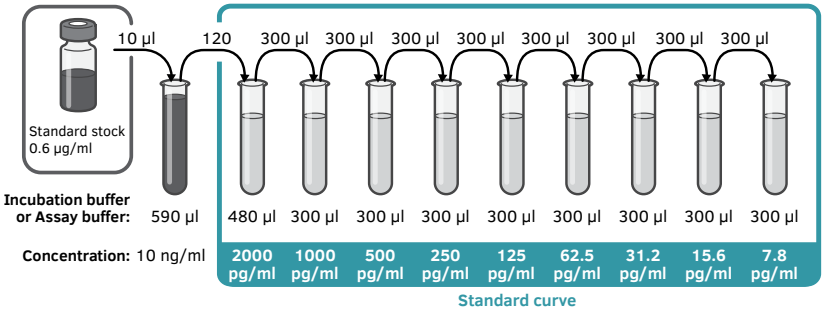
Analysis of serum/plasma requires the use of Assay buffer (product code: 3652-J2). The Assay buffer blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The Assay buffer should be used for dilution of standard, samples, and detection antibody.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.6 µg/ml by adding 0.7 ml of the standard reconstitution buffer. Allow the standard to dissolve for 5 minutes and mix thoroughly. The standard should be kept in aliquots at -20 °C. Avoid repeated freeze-thaw cycles.

Preparation of standard curve

Prepare within 30 minutes of use. Volumes are sufficient for duplicates.



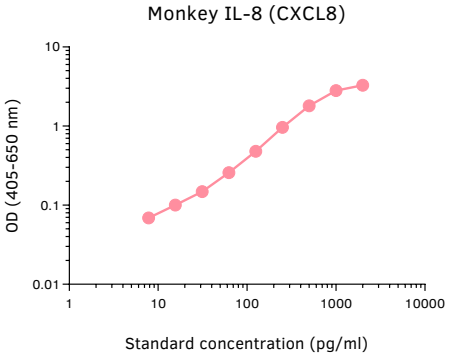
Protocol

Day 1

1. Add 100 µl/well of capture mAb MT8H6 diluted to 2 µg/ml in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 °C.

Day 2

2. Empty the plate and add 200 µl/well of PBS with 0.05% Tween 20 and 0.1% BSA (incubation buffer) to block the plate. Incubate for 1 hour at room temperature.
3. Wash the plate 5 times with PBS containing 0.05% Tween 20 (300 µl/well).
4. Add 100 µl/well of samples or standards diluted in incubation buffer or Assay buffer. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
5. Wash as above.
6. Add 100 µl/well of detection mAb 26E5-biotin diluted to 0.1 µg/ml in incubation buffer or Assay buffer. Incubate for 1 hour at room temperature.
7. Wash as above.
8. Add 100 µl/well of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
9. Wash as above.
10. Add 100 µl/well of pNPP substrate (product code: 3652-P10) and incubate at room temperature protected from direct light for approximately 60 minutes.
11. Measure the optical density in an ELISA reader at 405 nm. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.



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