Datasheet & Protocol

MABTECH

ELISA Flex: Monkey CCL4 (HRP)

3494M-1H-6 | 3494M-1H-20

ELISA Flex kit for quantitative determination of native and recombinant monkey CCL4 (MIP-1 β) in solution, e.g. cell supernatant.

The kit includes	3494M-1H-6 for 6 plates	3494M-1H-20 for 20 plates
Capture mAb: MTBN13	80 µl	250 µl
Detection mAb: MTBN3, biotin	150 μl	500 µl
Streptavidin-HRP	80 µl	250 µl
Recombinant human CCL4 ELISA standard	1 vial	1 vial
Standard reconstitution buffer A8	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-HRP is supplied in PBS 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 CCL4 (MIP-1 β). The mAbs cross-react with CCL4 (MIP-1 β) from non-human primates. Please visit www.mabtech.com for reactivity on NHP species.

Standard range 2-500 pg/ml

Calibration

No international standard exists for calibration.

Analysis of serum and plasma samples

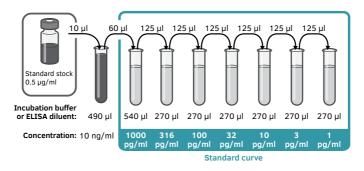
Analysis of serum/plasma requires the use of ELISA diluent (product code: 3652-D2). The ELISA diluent blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The ELISA diluent should be used for dilution of standard, samples, and detection antibody.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.5 μ g/ml by adding 1 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.



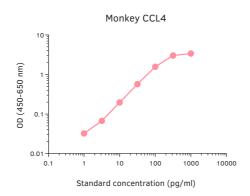
Protocol

Day 1

1. Add 100 μl/well of capture mAb MTBN13 diluted to 0.5 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 μ /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 μ l/well).
- **4.** Add 100 μl/well of samples or standards diluted in incubation buffer or ELISA diluent. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
- 5. Wash as above.
- **6.** Add 100 μ /well of detection mAb MTBN3-biotin diluted to 1 μ g/ml in incubation buffer or ELISA diluent. Incubate for 1 hour at room temperature.
- 7. Wash as above.
- **8.** Add 100 μl/well of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. Please note that sodium azide used in buffers will inhibit HRP activity.
- 9. Wash as above.
- **10.** Add 100 μl/well of TMB substrate (product code: 3652-F10) and incubate at room temperature, protected from direct light for 15 minutes.
- **11.** Add 100 μ l/well of 0.2 M H₂SO₄ to stop the reaction.
- **12.** Measure the optical density in an ELISA reader at 450 nm within 15 min. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.





Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.



The products are for research use only.

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