Datasheet & Protocol

MABTECH

ELISA Flex: Human GM-CSF (ALP)

3480-1A-6 | 3480-1A-20

ELISA Flex kit for quantitative determination of native and recombinant human GM-CSF in solution, e.g. cell supernatant and serum/plasma.

The kit includes	3480-1A-6 for 6 plates	3480-1A-20 for 20 plates
Capture mAb: 21C11 (1 mg/ml)	150 μl	500 μl
Detection mAb: 23B6, biotin (1 mg/ml)	80 µl	250 μl
Streptavidin-ALP	80 µl	250 μl
Recombinant human GM-CSF 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 GM-CSF. The mAbs cross-react with GM-CSF from non-human primates (NHP). Please visit www.mabtech.com for reactivity on NHP species.

Standard range

5-500 pg/ml

Calibration

The ELISA standard has been calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. One ng of supplied standard equals 15 U of 88/646 NIBSC-standard. Please note that the calibration is batch specific.

Analysis of serum and plasma samples

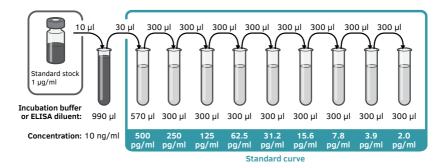
Analysis of serum/plasma requires the use of ELISA diluent (product code: 3652-D2). The ELISA diluent blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The ELISA diluent should be used for dilution of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

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 1 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.



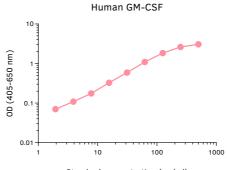
Protocol

Day 1

1. Add 100 μl/well of capture mAb 21C11 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 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 μl/well of detection mAb 23B6-biotin diluted to 1 μg/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-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.



Standard concentration (pg/ml)



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.

MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages there from.

Mabtech AB (Head Office) Sweden Tel: +46 8 716 27 00 mabtech@mabtech.com Mabtech, Inc. USA Tel: +1 513 871-4500 mabtech.usa@mabtech.com

www.mabtech.com