

ELISA Flex: Human Perforin (ALP)

3465-1A-6 | 3465-1A-20

ELISA Flex kit for quantitative determination of human Perforin in solution, e.g. cell supernatant and serum/plasma.

| The kit includes | 3465-1A-6 for 6 plates | 3465-1A-20 for 20 plates |
|---|-----------------------------------|-------------------------------------|
| Capture mAbs: Pf-80/164 (1 mg/ml) | 300 µl | 1000 µl |
| Detection mAb: Pf-344, biotin (1 mg/ml) | 80 µl | 250 µl |
| Streptavidin-ALP | 80 µl | 250 µl |
| Human Perforin 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 human perforin. The mAbs cross-react with perforin from non-human primates (NHP). Please visit www.mabtech.com for reactivity on NHP species.

Standard range

50-5000 pg/ml

Calibration

No international standard exists for calibration.

Analysis of serum and plasma samples

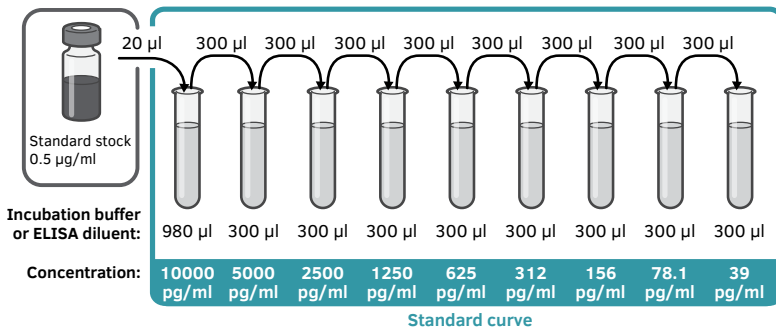
Analysis of serum/plasma requires the use of ELISA diluent (product code: 3652-D2). The ELISA diluent blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The ELISA diluent should be used for dilution of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

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.5 µg/ml by adding 0.5 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.



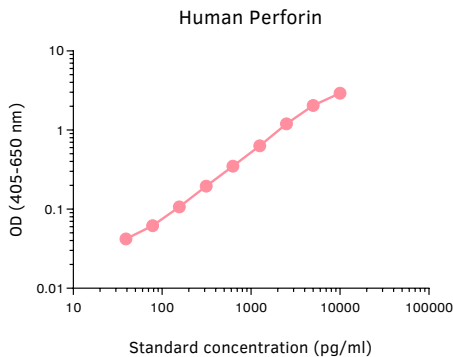
Protocol

Day 1

1. Add 100 μ l/well of capture mAb Pf-80/164 diluted to 4 μ g/ml in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 $^{\circ}$ C.

Day 2

2. Empty the plate and add 200 μ l/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 μ l/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 l/well of detection mAb Pf-344-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 μ l/well of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
9. Wash as above.
10. Add 100 μ l/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.



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



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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