

Human Perforin ELISA development kit

Product Code: 3465-1A-20

CONTENTS, development kit for 20 plates:

Vial 1 (yellow top)

Monoclonal antibody Pf-80/164, a combination of two different antibodies (1 ml)

Concentration: 1 mg/ml

Vial 2 (blue top)

Biotinylated monoclonal antibody Pf-344 (250 μ l)

Concentration: 1 mg/ml

Vial 3 (white top)

Streptavidin-Alkaline Phosphatase (250 μ l)

Vial 4

Lyophilised human Perforin standard (0.5 μ g)

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

STORAGE:

Shipped at ambient temperature. On arrival box 1 should be stored refrigerated at 4-8°C and box 2 should be stored frozen at -20°C.

General

Intended use: This assay is based on the use of a combination of two coating antibodies, Pf-80 and Pf-164, for optimal results. For quantitative determination of human Perforin in solution, e.g. cell culture supernatant. Also reacts with Perforin from rhesus and cynomolgus macaques.

Serum/plasma samples: Please note that determination of analyte in human serum/plasma samples by this kit requires the use of ELISA diluent (product code: 3652-D2) for dilution of samples, standard and detection antibody. The diluent prevents false positive read-outs which may be caused by interference of heterophilic antibodies commonly found in human plasma and serum. The ELISA diluent has been validated using serum/plasma from normal healthy human blood donors. Please note that heterophilic antibody interference in samples from human subjects with various diseases or other conditions has not been assessed. Please contact Mabtech for further information.

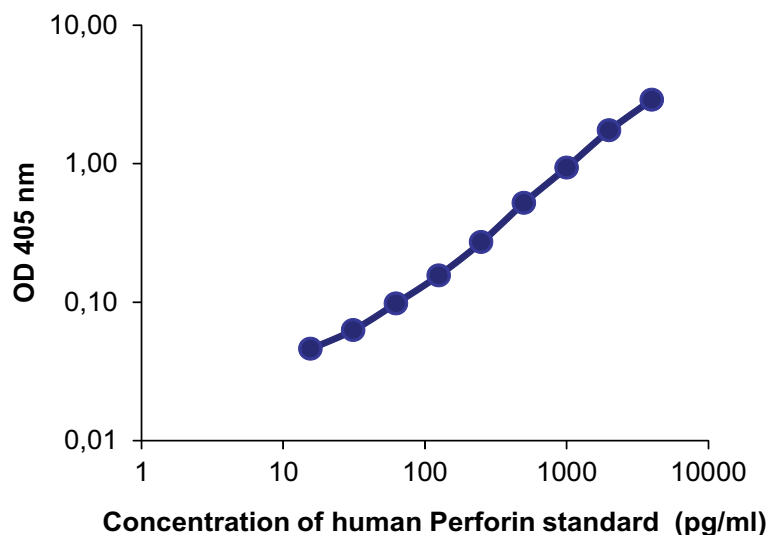
Reagents: Antibodies are supplied in sterile-filtered (0.2 μm) PBS with sodium azide (0.02%). Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG.

Standard range: 25-2500 pg/ml

Limit of detection: 12 pg/ml

Intra-assay variation: < 5%

Standard calibration: One vial of lyophilized standard contains 0.5 μg of human Perforin, estimated by comparison to purified reference Perforin. Calibration is batch specific.



Guidelines for Human Perforin ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb Pf-80/164, diluted to 4 µg/ml in PBS, pH 7.4, by adding 100 µl/well. Incubate overnight at 4-8°C.
- Day 2**
2. Wash twice with PBS (200 µl/well).
 3. Block plate by adding 200 µl/well of PBS with 0.05% Tween 20 containing 0.1% BSA (incubation buffer). Incubate for 1 hour at room temperature.
 4. Wash five times with PBS containing 0.05% Tween.
 5. Prepare standard by reconstituting contents of vial 4 in 1 ml PBS water. Leave at room temperature for 15 minutes and then vortex the tube. This gives a stock solution of 0.5 µg/ml which should be used immediately or stored in aliquots at -20°C for future use. We recommend the aliquots not to be refrozen after initial use. For the test, prepare dilutions of the stock using the standard range as a guideline.
 6. Add 100 µl/well of samples or standards diluted in incubation buffer or ELISA diluent for serum/plasma samples and incubate for 2 hours at room temperature.
 7. Wash as in step 4.
 8. Add 100 µl/well of mAb Pf-344-biotin at 1 µg/ml in incubation buffer or ELISA diluent for serum/plasma samples. Incubate for 1 hour at room temperature.
 9. Wash as in step 4.
 10. Add 100 µl/well of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
 11. Wash as in step 4.
 12. Add 100 µl/well of appropriate substrate solution e.g. p-nitrophenyl-phosphate (pNPP), available from Mabtech product code 3652-P10.
 13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

References:

Zuber B, et al. Detection of human perforin by ELISpot and ELISA: ex vivo identification of virus-specific cells. *J. Immunol. Methods*, 302: 1, 2005.

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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