

ELISA for Human Perforin

Product Code: 3465-1H-6

CONTENTS, development kit for 6 plates:

Vial 1 (yellow top)

Monoclonal antibody Pf-80/164, a combination of two different antibodies (300 μ l)

Concentration: 1 mg/ml

Vial 2 (blue top)

Biotinylated monoclonal antibody Pf-344 (80 μ l)

Concentration: 1 mg/ml

Vial 3 (white top)

Streptavidin-Horseradish Peroxidase (80 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.

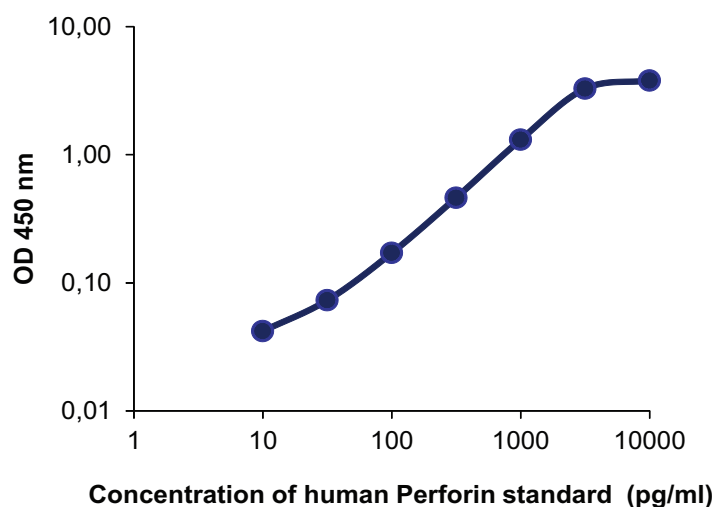
Reagents: Antibodies are supplied in sterile-filtered (0.2 µm) PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 1% BSA and 0.15% Kathon CG.

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. 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-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.**
 11. Wash as in step 4.
 12. Add 100 µl/well of appropriate substrate solution e.g.
 13. Measure the optical density in an ELISA reader after suitable developing time. If required stop the reaction first.

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.

NOTE; 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 therefrom.



MABTECH AB
Box 1233
SE-131 28 Nacka Strand
Sweden
Tel: +46 8 716 27 00
Fax: +46 8 716 27 01
E-mail: mabtech@mabtech.com
www.mabtech.com

MABTECH Inc
M.E.B. 220
3814 West Street
Cincinnati, OH 45227
USA
Tel: +1 513 871 4500
Fax: +1 513 871 7353
E-mail: mabtech.usa@mabtech.com

MABTECH AB Büro Deutschland
Germany
Tel: +49 40 4135 7935
Fax: +49 40 4135 7945
E-mail: mabtech.de@mabtech.com

2013-01-24

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the following standards:



MABTECH AUSTRALIA Pty Ltd
resolvingIMAGES
Unit 22, 196 Settlement Road
Thomastown Victoria 3074
Australia
Tel: +61 3 9466 4007
Fax: +61 3 9466 4003
E-mail: mabtech.au@mabtech.com

MABTECH AB Bureau de liaison France
BP 255, 1300 route des Crêtes
06905 Sophia Antipolis
France
Tel: +33 (0)4 92 38 80 70
Fax: +33 (0)4 92 38 80 71
E-mail: mabtech.fr@mabtech.com