

# ELISA for Human Granzyme B

Product Code: 3485-1H-20

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CONTENTS, development kit for 20 plates:

**Vial 1 (red top)**

Monoclonal antibody GB10 (500  $\mu$ l)

Concentration: 1 mg/ml

**Vial 2 (green top)**

Biotinylated monoclonal antibody GB11 (250  $\mu$ l)

Concentration: 1 mg/ml

**Vial 3 (white top)**

Streptavidin-Horseradish Peroxidase (250  $\mu$ l)

**Vial 4**

Granzyme B standard (693 ng)

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:** For quantitative determination of human Granzyme B in solution, e.g. cell culture supernatant.

**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.

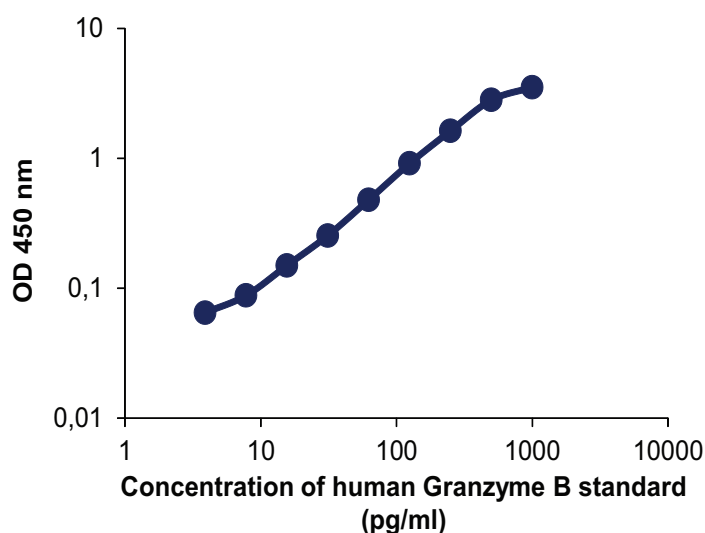
**Reagents:** Antibodies are supplied in sterile-filtered (0.2  $\mu\text{m}$ ) PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 1% BSA and 0.15% Kathon CG.

**Standard range:** 7-700 pg/ml

**Limit of detection:** 4 pg/ml

**Intra-assay variation:** < 5%

**Standard calibration:** One vial of lyophilized YT-supernatant contains 693 ng human Granzyme B, estimated by comparison to purified reference Granzyme B. Calibration is batch-specific.



# Guidelines for Human Granzyme B ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb GB10, diluted to 2  $\mu\text{g}/\text{ml}$  in PBS, pH 7.4, by adding 100  $\mu\text{l}/\text{well}$ . Incubate overnight at 4-8°C.
- Day 2**
2. Wash twice with PBS (200  $\mu\text{l}/\text{well}$ ).
  3. Block plate by adding 200  $\mu\text{l}/\text{well}$  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 1ml pure water. This gives a stock solution of 693 ng/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  $\mu\text{l}/\text{well}$  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  $\mu\text{l}/\text{well}$  of mAb GB11-biotin at 1  $\mu\text{g}/\text{ml}$  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  $\mu\text{l}/\text{well}$  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  $\mu\text{l}/\text{well}$  of appropriate substrate solution.
  13. Measure the optical density in an ELISA reader after suitable developing time. If required stop the reaction first.

**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.



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



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