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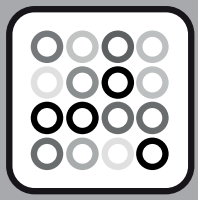
Human Granzyme B ELISA^{PRO} kit

3486-1HP-1 | 3486-1HP-2 | 3486-1HP-10

Datasheet & Protocol



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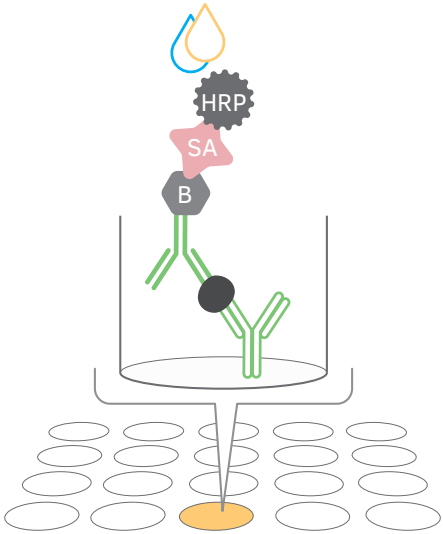
Introduction

Mabtech's carefully validated ELISA^{PRO} kits provide all the necessary reagents to conveniently quantify analytes in serum, plasma, and cell culture supernatants in a robust, sensitive, and specific manner.

ELISA assay principle

ELISA^{PRO} kits are supplied with ELISA strip plates precoated with monoclonal antibody (mAb). Analyte in the sample is captured by the coated mAb and detected by the biotinylated detection mAb followed by Streptavidin-HRP (SA-HRP). Addition of TMB substrate will result in a colored substrate product. The reaction is stopped with sulfuric acid and the optical density can be quantified using an ELISA plate reader. The concentration of analyte is determined by comparison to a serial dilution of the ELISA standard analyzed in parallel.



Analysis of serum and plasma samples

The ELISA^{PRO} kits include ELISA diluent, a buffer that prevents false-positive signals. The buffer blocks heterophilic antibodies from cross-linking the assay antibodies. Heterophilic antibodies are commonly found in human serum/plasma and can also be present in other species. The buffer has been validated using serum/plasma samples from healthy human blood donors.

Shipping and storage

The kit is shipped at ambient temperature. All reagents should be stored at 2-8 °C upon receipt, except the standard which should be stored at -20 °C. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use. Do not combine components from different kit batches or components from other suppliers.

Contents

Component	1-plate kit	2-plate kit	10-plate kit
Pre-coated ELISA strip plate: Anti-Granzyme B mAb MT34B6	1 x 96 wells	2 x 96 wells	10 x 96 wells
Recombinant human Granzyme B ELISA standard	1 vial	1 vial	1 vial
Detection mAb MT28, biotinylated (0.5 mg/ml)	25 µl	50 µl	250 µl
Streptavidin-HRP (1000x)	15 µl	25 µl	125 µl
Standard reconstitution buffer A5	1 ml	1 ml	1 ml
Wash buffer concentrate (20x)	120 ml	120 ml	5 x 120 ml
ELISA diluent	120 ml	120 ml	3 x 120 ml
Streptavidin-HRP diluent	15 ml	25 ml	120 ml
TMB substrate	15 ml	25 ml	120 ml
Stop solution	15 ml	25 ml	120 ml
Adhesive plate covers	3	6	30

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

Materials required but not supplied

- Microplate reader capable of reading at 450 nm
- ELISA plate washer; automated or manual (e.g., multipipette or squirt bottle)
- Precision pipettes, tips, and graduated cylinders
- Tubes for standard and sample dilutions
- Distilled or deionized water

Safety information

The Stop solution, 0.18 M H_2SO_4 (< 1%), is irritating to eyes and skin and should be handled with care. The standard should also be handled carefully as the effects of exposure are unknown. Buffers and reagents in solution contain the preservative Kathon CG (0.002%), a potential allergen that may cause sensitization through skin contact. Human and animal samples should be treated as potentially hazardous biologic material. All material should be disposed of in accordance with local regulations. For further information please consult the Safety Data Sheet on our website.

Preparation

- Allow the plates and assay reagents to reach room temperature before starting the assay (except for the TMB substrate which should preferably be used cold).
- Plan the plate layout to include a standard curve, samples, and an assay background control all in duplicate. The volume per well should not exceed 100 µl. Plate blanks (only substrate and Stop solution) can be included for subtraction by the reader software.

Wash buffer

Add 50 ml Wash buffer concentrate to 950 ml distilled or deionized water (sufficient for all washing steps of 1 plate). If crystals have formed in the 20x concentrate, bring to room temperature and mix gently to dissolve.

Samples

For accurate assay performance, all samples should be diluted at least 2-fold in ELISA diluent. Samples can be diluted in polypropylene tubes or plates; buffer should be added before the samples and visible precipitates should be removed. The use of strongly hemolyzed and hyperlipemic samples may result in inaccurate determination of the concentration.

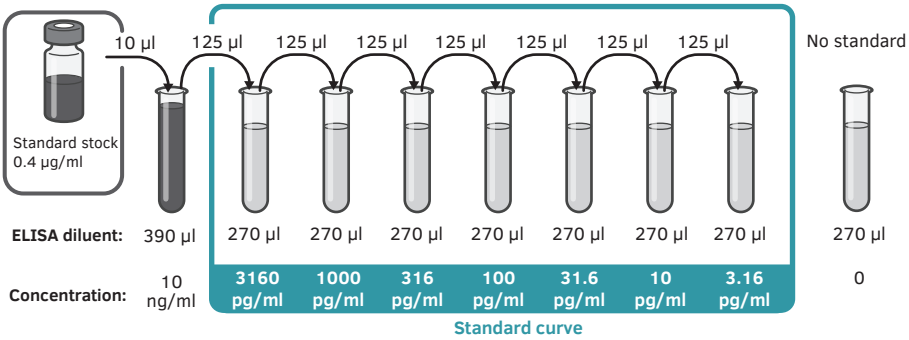
Samples containing high levels of analyte exceeding the standard range of the assay will require further dilution.

ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.4 µg/ml by adding 0.5 ml 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

Dilute the standard stock solution to create a standard curve as shown. The indicated volumes are sufficient for duplicates. The last vial is used as an assay background control, i.e., the standard should be omitted. Prepare the standard curve within 30 minutes of use.



Detection antibody

Dilute the detection antibody in ELISA diluent to 1 µg/ml within 15 minutes of use. For each plate, dilute 24 µl detection antibody in 12 ml ELISA diluent.

Streptavidin-HRP

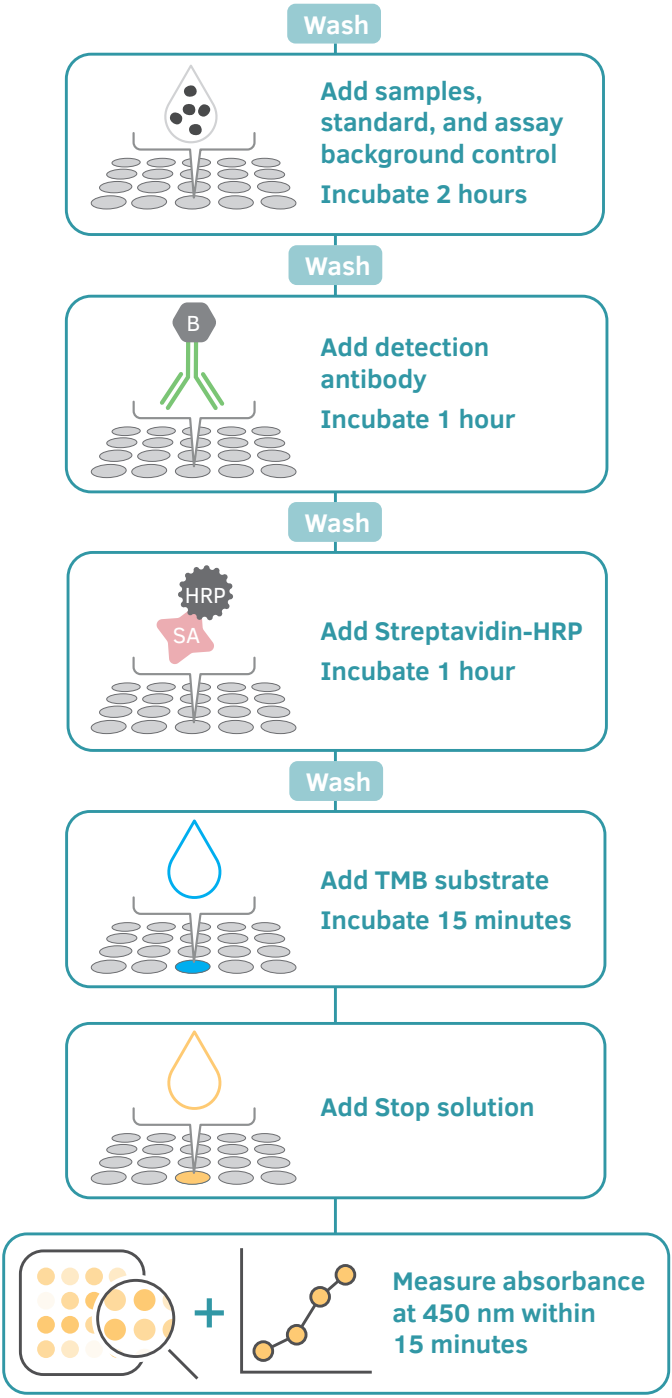
Dilute the Streptavidin-HRP 1000-fold in Streptavidin-HRP diluent within 15 minutes of use. For each plate, dilute 12 µl Streptavidin-HRP in 12 ml Streptavidin-HRP diluent.

Protocol

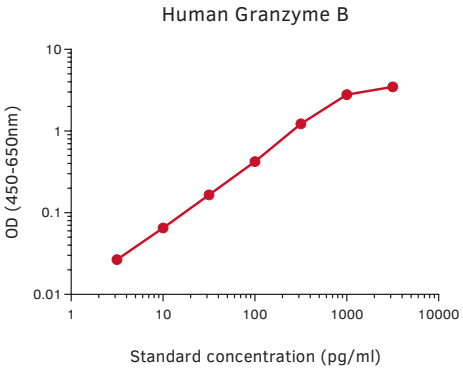
Prepare the reagents, standard curve, and samples as described in the Preparation section. Assemble the required number of strips in the plate frame and label the top of each strip. Store the remaining strips in the foil bag containing the desiccant at 4-8 °C.

1. Wash the plate 5 times with wash buffer (300 µl/well). After the final wash, invert and tap the plate firmly against absorbent paper. Immediately proceed to the next step.
2. Add samples (diluted at least 2-fold), standard, and assay background control (100 µl/well). Mix by tapping the plate. Cover the plate with an adhesive plate cover and incubate at room temperature for 2 hours.
3. Wash the plate as described above.
4. Add detection antibody (100 µl/well). Cover the plate and incubate at room temperature for 1 hour.
5. Wash the plate as described above.
6. Add Streptavidin-HRP (100 µl/well). Cover the plate and incubate at room temperature for 1 hour.
7. Wash the plate as described above.
8. Add TMB substrate (100 µl/well). Incubate at room temperature protected from direct light for 15 minutes.
9. Add Stop solution to all wells (100 µl/well) to stop the color development.
10. Measure absorbance at 450 nm within 15 minutes. If possible, use a reader capable of subtracting a reference wavelength of between 570 and 650 nm.

    We recommend the use of ELISA software utilizing a 4- or 5-parameter curve fit. Subtract the mean absorbance value of the blank from the standard, background control, and samples prior to creating the standard curve.


Wash
Add samples, standard, and assay background control
Incubate 2 hours
Wash
B
Add detection antibody
Incubate 1 hour
Wash
HRP
SA
Add Streptavidin-HRP
Incubate 1 hour
Wash
Add TMB substrate
Incubate 15 minutes
Add Stop solution
Measure absorbance at 450 nm within 15 minutes

Performance



Representative standard curve

Standard range 10-3160 pg/ml

Sensitivity 5 pg/ml

The lowest concentration that can be detected, but not necessarily quantified with precision and accuracy. This was determined by adding 4 standard deviations to the mean OD of background wells.

Calibration

No international standard exists for calibration.

Precision

	Intra-assay			Inter-assay		
Sample	1	2	3	1	2	3
n	10	10	10	4	4	4
Mean (pg/ml)	438.2	158.4	61.9	418.9	156.9	62.0
SD	18.7	6.0	4.0	20.0	1.3	0.5
CV%	4.3	3.8	6.5	4.8	0.8	0.9

Intra-assay and inter-assay precision were determined at 3 different concentrations of analyte (4 assays with 10 replicates/concentration).

Recovery

	Spike concentration (pg/ml)	Average recovery % (range)
Plasma	450	89 (83-93)
	150	88 (85-94)
	50	87 (84-98)

Three concentrations of standard were spiked in a human plasma pool. Five replicates/concentration were tested in 4 assays.

Linearity

Recovery of a high concentration of standard in human plasma after serial dilution in ELISA diluent (1:2 to 1:8) ranged from 82% to 105% with a mean recovery of 96%.

Specificity

The ELISA^{PRO} kit contains a matched pair of mAbs specific for native and recombinant human Granzyme B.

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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.

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