

ELISA Pro: Mouse ApoE

3752-1HP-1 | 3752-1HP-2 | 3752-1HP-10

Datasheet & Protocol



Table of Contents

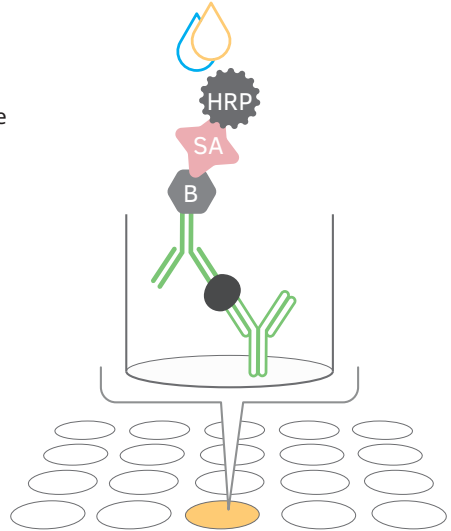
Introduction	4
Shipping and storage	4
Contents.....	5
Preparation.....	6
Protocol	8
Performance.....	10

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. For Research Use Only (RUO). Not for use in diagnostic procedures.

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.



Shipping and storage

The kit is 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. 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-apoE mAb mE1	1 x 96 wells	2 x 96 wells	10 x 96 wells
Purified mouse apoE ELISA standard	1 vial	1 vial	2 vials
Detection mAb mE29, biotin (0.5 mg/ml)	25 µl	50 µl	250 µl
Streptavidin-HRP	15 µl	25 µl	125 µl
Standard reconstitution buffer A8	1 ml	1 ml	2 x 1 ml
Wash buffer concentrate (20x)	120 ml	120 ml	5 x 120 ml
Apo ELISA buffer concentrate (5x)	60 ml	60 ml	5 x 60 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 H₂SO₄ (< 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 . Include a plate blank (wells with only Substrate and Stop solution) to be used for subtraction before analysis.

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.

Apo ELISA buffer

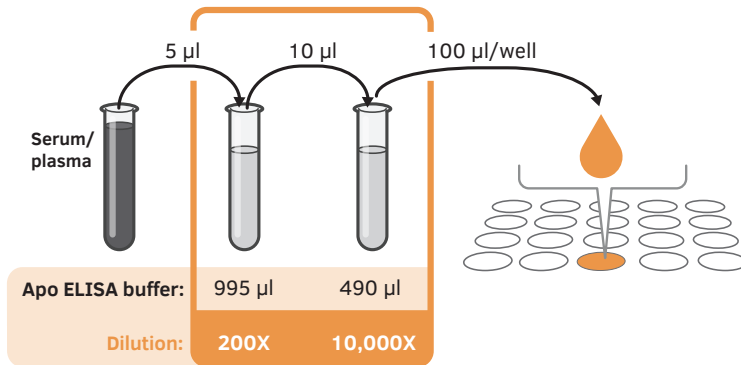
Prepare the required volume of Apo ELISA buffer by diluting Apo ELISA buffer concentrate 5-fold with distilled or deionized water. For each plate, add 30 ml Apo ELISA buffer concentrate to 120 ml water.

Samples

All samples should be diluted at least 2-fold in Apo ELISA buffer. Remove visible precipitates and dilute in tubes/plates, buffer should be added prior to the samples. Strongly hemolyzed and hyperlipemic samples may give inaccurate quantifications. Samples containing high levels of analyte exceeding the standard range of the assay will require further dilution.

Dilution guidelines for mouse serum/plasma

We recommend a dilution factor of 10,000X based on repeated analyses of BALB/c and C57BL/6 samples. Precise pipetting is important, change tips between dilution steps and use freshly made dilutions. Indicated volumes are sufficient for duplicates.

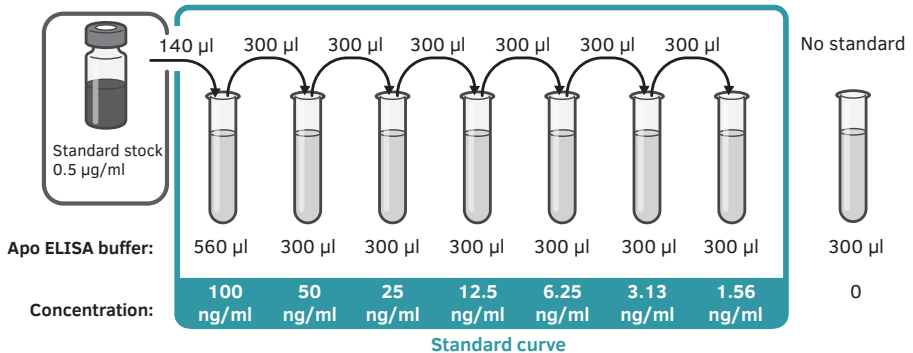


ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.5 µg/ml by adding 1 ml Standard reconstitution buffer. Do not stir. Allow the standard to dissolve for 20 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 mAb in Apo ELISA buffer to a concentration of 1 µg/ml within 15 minutes of use. For each plate, add 24 µl detection mAb to 12 ml Apo ELISA buffer.

Streptavidin-HRP

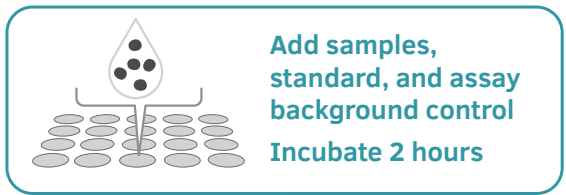
Dilute the Streptavidin-HRP 1:1000 in Streptavidin-HRP diluent within 15 minutes of use. For each plate, add 12 µl Streptavidin-HRP to 12 ml Streptavidin-HRP diluent.

Protocol

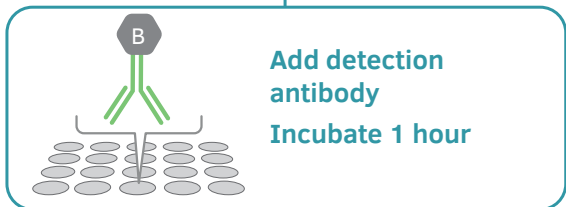
Prepare the reagents, standard curve, and samples as described in the Preparation section. Assemble the required number of strips in the plate frame and label the top of each strip. Store the remaining strips in the foil bag containing the desiccant at 4-8 °C.

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

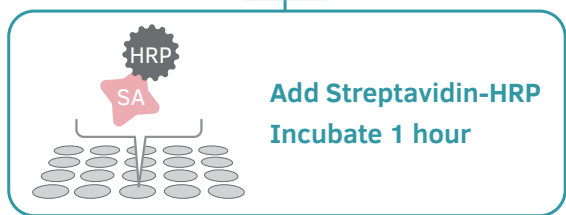
We recommend the use of an ELISA software utilizing a 4- or 5-parameter curve fit. Subtract the mean absorbance value of the blank from the samples, standard and assay background control prior to creating the standard curve and analyzing the results.



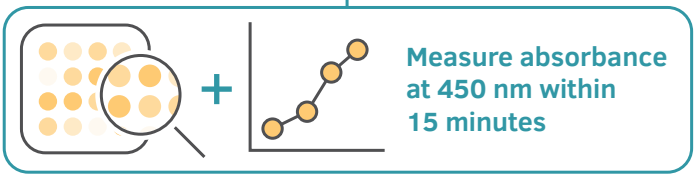
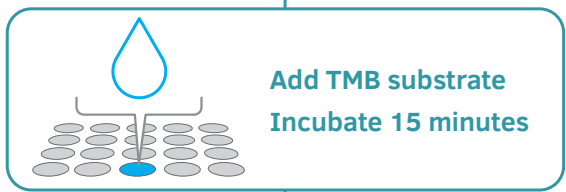
Wash



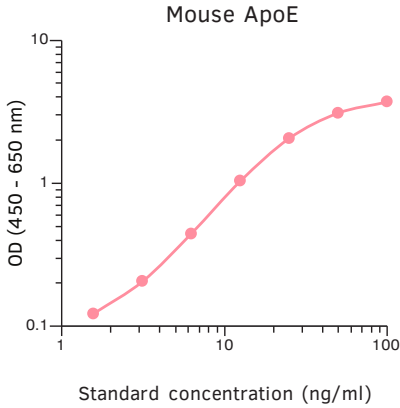
Wash



Wash



Performance



Representative standard curve

Standard range 1.56-100 ng/ml

Sensitivity 1.0 ng/ml

The lowest concentration that can be detected, but not necessarily quantified with precision and accuracy. This was determined by adding 3 standard deviations to the mean OD of background wells.

Calibration

No international standard exists for calibration.

Precision

Sample	Intra-assay			Inter-assay		
	1	2	3	1	2	3
n	8	8	8	3	3	3
Mean (ng/ml)	20.1	10.1	5.3	21.1	10.3	5.5
SD	0.7	0.4	0.1	2.9	0.7	0.2
CV%	3.5	3.7	1.9	13.8	7.1	3.5

Intra-assay and inter-assay precision were determined at 3 different concentrations of analyte (8 replicates per concentration in 3 assays).

Linearity

Dilution of a mouse plasma gives a mid-curve recovery of 98-110% in repeated experiments (mean 102%).

Specificity

The kit is based on a matched pair of mAbs specific for mouse ApoE.

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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