ELISA^{PRO} kit
for Human Latent TGF-β1

Kit for 2 plates

Product code: 3550-1HP-2

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KIT DESCRIPTION

This ELISA^{PRO} kit is a complete kit for the quantification of human latent TGF-β1 in biological fluids such as plasma and cell culture supernatants. Analysis of latent TGF-β1 by this kit does not require any pre-treatment of samples to dissociate the latent complex. The ELISA detects the Latency Associated Protein (LAP), which is a part of the latent TGF-β1 complex, but does not detect human latent TGF-β2 or -β3 or bovine latent TGF-β1. Samples are added to plates pre-coated with a monoclonal antibody (mAb). Captured cytokine is detected by adding a biotinylated mAb followed by streptavidin-horseradish peroxidase (SA-HRP). Addition of TMB substrate will result in a colored product with an intensity directly proportional to the concentration of cytokine in the sample. The cytokine concentration is determined by comparison to a serial dilution of standard analyzed in parallel.

REAGENTS PROVIDED

Component	Quantity	Description/comments
Precoated 96-well strip plate (12 strips x 8 wells)	2 plates	Plate coated with anti-Human Latent TGF-β1. Supplied in foil bag with desiccant.
Cytokine standard	1 vial	Recombinant human LAP. See "Preparations" for reconstitution and dilution.
Detection antibody	1 vial (50 µl)	Biotinylated anti-human latent TGF-β1 mAb (0.5 mg/ml). Dilute before use.
SA-HRP	1 vial (30 µl)	Streptavidin-horseradish peroxidase conjugate. Dilute before use.
Standard reconstitution buffer A8 (ready-to-use)	1 vial (1ml)	For reconstitution of lyophilized cytokine standard.
Wash buffer concentrate (20x)	1 bottle (120 ml)	For all wash steps. Dilute before use.
ELISA diluent/ Assay buffer (ready-to-use)	1 bottle (120 ml)	Protein-containing buffer for: 1) dilution of all samples; 2) serial dilution of standard; 3) dilution of detection antibody.
SA-HRP diluent (ready-to-use)	1 bottle (25 ml)	For dilution of SA-HRP.
TMB substrate (ready-to-use)	1 bottle (25 ml)	3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution containing hydrogen peroxide.
Stop solution (ready-to-use)	1 bottle (25 ml)	1 M H_2SO_4
Adhesive plate covers	6	To cover plates during incubations.

To ensure total recovery of stated quantity, bottles and vials are filled with larger volume than indicated.

STORAGE AND EXPIRY DATE

Shipped at ambient temperature. On arrival all components of the kit should be stored at 2-8°C. After reconstitution of the lyophilized standard, it should be aliquoted and kept at -20 °C. The expiry date for the unopened kit is indicated on the box. We recommend to use opened kit components within one month.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Microplate reader capable of reading at 450 nm; preferably able of subtracting a reference wavelength between 570-650 nm.
- ELISA plate washer; automatic (adaptable for ELISA strip plates) or manual (e.g. multipipette or squirt bottle).
- Precision pipettes and tips.
- Beakers, flasks and graduated cylinders necessary for reagent preparations.
- Tubes for standard and sample dilutions.
- Distilled or deionized water.

SAFETY ISSUES

- The stop solution (1 M H_2SO_4) is irritating to eyes and skin and should be handled with care.
- The cytokine standard should be handled with care due to unknown effects of exposure.
- Buffers and reagents in solution contain 0.15% of the preservative Kathon CG, a potential contact allergen which may cause sensitization by skin contact.
- Human and animal samples should be treated as potentially hazardous biological material.
- All material and samples should be disposed of in accordance with local regulations.

PROCEDURAL NOTES

- Do not combine components from different kit lots or components from other suppliers.
- Dilutions of standard and samples can be prepared in plastic or glass tubes.
- Total sample or standard volume added per well should not exceed 100 µl.
- The use of duplicates for each dilution of the standard, samples and blank is recommended.
- Prior to adding new reagents to the wells, ensure that there is no residual wash buffer remaining in the wells. The wells can be emptied by turning the plate upside down followed by gently tapping the plate against clean tissue paper. Please be careful to avoid that the strips fall out of the frame e.g. grip the plate by the middle.
- Plasma components present in the sample may have an impact on the performance of the assay. For this reason, all samples should be diluted at least 2x in "ELISA diluent".
- Sodium azide and other nucleophilic substances (often used as preservative in various buffers) interfere with the activity of horseradish peroxidase. Thus, avoid the use of other wash buffers or solutions that may contain such interfering substances.

PREPARATIONS

Plates

Allow the plates to adjust to room temperature (18-25°C) before opening the bags. Plan the experiment to include a standard curve, assay background control, blank and sample wells. To the blank wells, only "TMB substrate" and "Stop solution" should be added. Assemble the required numbers of strips in the plate frame. The strips used for the experiment can be marked e.g. with a marker pen. Store the remaining strips in the foil bag containing the desiccant at 4-8°C.

Wash buffer

Prepare the required volume of wash buffer by diluting "Wash buffer concentrate" 20x with distilled or deionized water. For 1 plate, prepare 1000 ml wash buffer by adding 50 ml "Wash buffer concentrate" to 950 ml distilled or deionized water.

ELISA standard

Recombinant human LAP (homodimer) is used as standard. Since it differs in molecular weight from latent TGF-β1, determination of latent TGF-β1 using the standard curve is based on a molar comparison. Reconstitute the standard to a stock solution of 120 nM by adding 1 ml of "Standard reconstitution buffer A8". Allow the standard to dissolve for 5 min, then mix thoroughly and aliquot. Store at -20°C. Avoid repeated freeze-thaw cycles of the aliquotes.

Preparation of standard curve

The standard curve can be prepared from cytokine standard stock solution just reconstituted or from thawed aliquotes. Prepare a serial dilution of the standard no more than 30 min prior to the experiment. Duplicate wells for the standard are recommended. Dilute the standard stock solution to create a standard curve ranging from 0.1-100 pM according to the scheme below. For the assay background control (0 pM), use only "ELISA diluent".

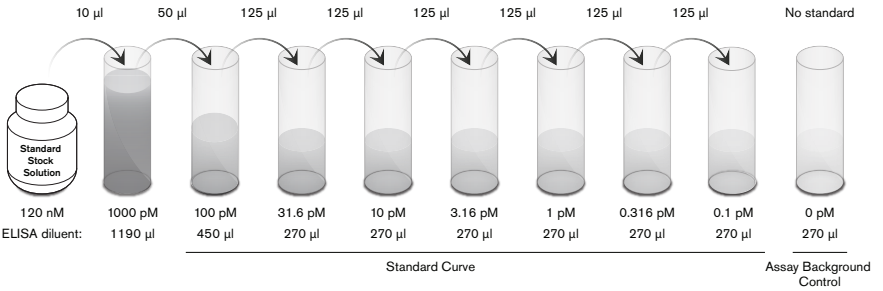

Figure 1. Recommended serial dilution of cytokine standard. The volumes indicated are sufficient for duplicates. The last vial should be 0 pM of standard i.e. the analyte should be omitted.

Samples

Dilute all samples in "ELISA diluent" prior to use. Analysis of latent TGF-β1 by this ELISA does not require any pre-treatment of samples to dissociate the latent compex. For quantification of latent TGF-β1 in blood, the use of plasma is recommended since serum contains high levels of latent TGF-β1 released from platelets during sample preparation. Plasma can be obtained using EDTA, citrate or heparin as anti-coagulants. To minimize the platelet content in the sample, an additional centrifugation of plasma at 10,000 x g for 10 min is recommended.

Important! All samples should be diluted at least 2x in "ELISA diluent". This applies also for samples where low analyte levels are anticipated. Samples containing high levels of cytokine exceeding the standard range of the assay will require further dilution. Sample dilutions can be made either in tubes or directly in the plate.

Detection antibody

Dilute the "Detection antibody" 500x in "ELISA diluent" prior to use. For 1 plate, dilute 24 µl "Detection antibody" in 12 ml "ELISA diluent" which will give a final "Detection antibody" concentration of 1 µg/ml.

SA-HRP

Dilute the "SA-HRP" 1000x in "SA-HRP diluent" prior to use. For 1 plate, dilute 12 µl "SA-HRP" in 12 ml "SA-HRP diluent".

ASSAY PROCEDURE

Throughout the assay all reagents and samples, except the "TMB substrate", should be adjusted to room temperature (18-25°C) prior to use. The "TMB substrate" should preferably be used cold.

1. Assemble the numbers of strips required for the standard curve, assay background control, the blanks and samples in the plate frame. The use of duplicates is recommended.

2. Wash the strips with 5x300 µl/well of diluted wash buffer. The wash buffer should be thoroughly removed in immediate relation to the next step.

3. Add 100 µl/well of each concentration of the diluted cytokine standard and assay background control. For the samples, add either 100 µl/well of pre-diluted sample or add 50 µl "ELISA diluent" + 50 µl sample per well. Mix by tapping the plate.

Important! Both the serial dilution of the cytokine standard and sample dilutions should be made in "ELISA diluent". Leave the blank wells empty. Cover the plate with adhesive plate cover and incubate at room temperature for 2 h.

4. Wash the wells as in step 2.

5. Add 100 µl per well of "Detection antibody", diluted 500x to a final concentration of 1 µg/ml in "ELISA diluent". Leave the blank wells empty. Cover the plate with adhesive plate cover and incubate at room temp for 60 min.

6. Wash the wells as in step 2.

7. Add 100 µl/well of "SA-HRP" diluted 1000x in "SA-HRP diluent". Leave the blank wells empty. Cover the plate and incubate at room temp for 60 min.

8. Wash the wells as in step 2.

9. Develop by adding 100 µl of "TMB substrate" to all wells (including the blank wells) and incubate at room temp in the dark for 15 min.

10. Stop the color development by adding 100 µl of "Stop solution" to all wells (including the blank wells).

11. Measure the absorbance at 450 nm in a microplate reader within 15 min of the addition of the "Stop solution". If possible, use a reader capable of subtracting a reference wavelength between 570-650 nm.

12. The use of ELISA software utilizing e.g. a 4-parameter curve fitting program is recommended for the data analysis. Subtract the mean absorbance value of the blank from the standard, the assay background control and the sample values prior to creating the standard curve and determining the cytokine concentrations in the samples.

PERFORMANCE OF THE ASSAY

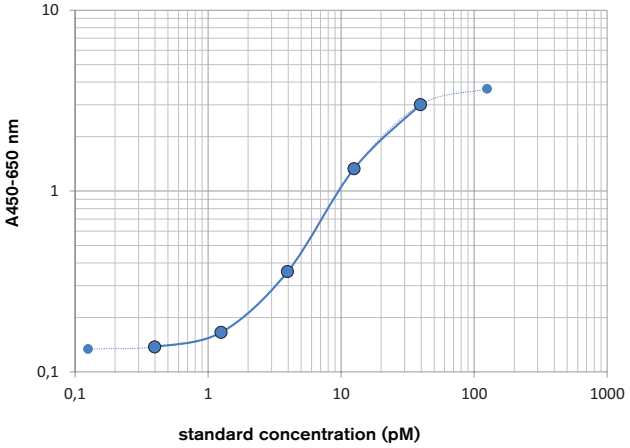

Figure 2. Display of the standard curve.

Standard range: 0.4-40 pM.
The standard range is the range in which determinations of analyte concentration can be done with precision, accuracy and linearity.

Recombinant human LAP (homodimer) is used as standard. Since it differs in molecular weight from latent TGF-β1, determination of latent TGF-β1 using the standard curve is based on a molar comparison. A concentration of 1 pM LAP corresponds to 1 pM latent TGF-β1. For conversion from pM to pg/ml: 1 pM LAP = 54 pg/ml and 1 pM latent TGF-β1 = 80 pg/ml. Note that cytokine values obtained should be multiplied with the dilution factor used for each sample.

Sensitivity: The limit of detection of this assay is 0.13 pM. It is the lowest concentration that is possible to detect but not necessarily quantify with precision and accuracy.

Dilution recovery: Dilution of plasma samples give a mid-curve recovery of 100% in repeated experiments.

Precision: The intraassay variation is 3.0%(CV). The interassay variation is 10.4%(CV).

LIMITATIONS OF THE ASSAY

Analysis of samples with high cytokine content
The standard curve should not be extrapolated beyond the recommended standard range as these parts of the standard curve are non-linear. Samples yielding absorbance values exceeding the highest point of the standard range should be re-analyzed at a higher dilution.

Aberrant sera and plasma
The use of strongly hemolyzed and hyperlipemic serum and plasma samples may result in erroneous determinations of cytokine concentrations.

Heterophilic antibodies in human serum and plasma
Heterophilic antibodies found in human serum/plasma are capable of binding to both the capture and detection antibodies used in capture ELISA. Heterophilic antibodies are found in a majority of human individuals and can, by cross-linking the assay antibodies used, result in false positive signals in capture ELISA. The "ELISA diluent" provided and used for dilution of samples prevents the heterophilic antibodies from cross-linking the capture and detection mAbs. The cytokine content of serum/plasma samples can therefore be measured without interference by heterophilic antibodies. The lack of interference by heterophilic antibodies in this MABTECH kit has been validated using plasma/serum samples from normal healthy human blood donors. Please note that heterophilic antibody interference in samples from human subjects with various diseases or other conditions have not been assessed.

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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.
MEB, Suite 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 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

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

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

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