Mouse IFN-γ ELISA^{PRO} kit

Kit for 2 plates

Product code: 3321-1HP-2

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KIT DESCRIPTION

Mabtech's enzyme-linked immunosorbent assay, ELISA^{PRO}, is a complete kit for the quantification of mouse IFN-γ in biological fluids such as serum, plasma and cell culture supernatants. The assay utilizes ELISA strip plates pre-coated with a capture monoclonal antibody (mAb), to which samples are added. Captured cytokine is detected by adding a biotinylated mAb followed by streptavidin-horseradish peroxidase (SA-HRP). Addition of the enzyme substrate TMB will result in a colored substrate product with an intensity that is directly proportional to the concentration of cytokine in the sample. The concentration of the cytokine in the sample is determined by comparison to a serial dilution of recombinant cytokine 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-Mouse IFN-γ. Supplied in foil bag with desiccant.
Cytokine standard	1 vial	Lyophilized recombinant mouse IFN-γ. See "Preparations" for reconstitution and dilution.
Detection antibody	1 vial (30 µl)	Biotinylated anti-mouse IFN-γ mAb (1 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)	0.18 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, with the exception of the lyophilized standard, should be stored at 2-8°C.**Please note that the lyophilized cytokine standard should be kept at -20°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, 0.18 M H_2SO_4 (< 1%), 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 0.002% 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.
- Serum/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 and an assay background control (9 x 2 wells), blank (2 wells) and sample wells. To the blank wells, only "TMB substrate" and "Stop solution" should be added. Before the analysis of absorbance values, the mean value of the blank wells should be subtracted from the standard, the assay background control and the sample values.

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
Reconstitute the cytokine standard to a standard stock solution of 1 µg/ml by adding 1 ml of "Standard reconstitution buffer A8" and allow the standard to dissolve for 5 min. Mix thoroughly and aliquot. Store at -20°C. Avoid repeated freeze-thaw cycles of the standard aliquotes.

Preparation of standard curve
The standard curve can be made 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 1-3160 pg/ml according to the scheme below. For the assay background control (0 pg/ml), use only "ELISA diluent".

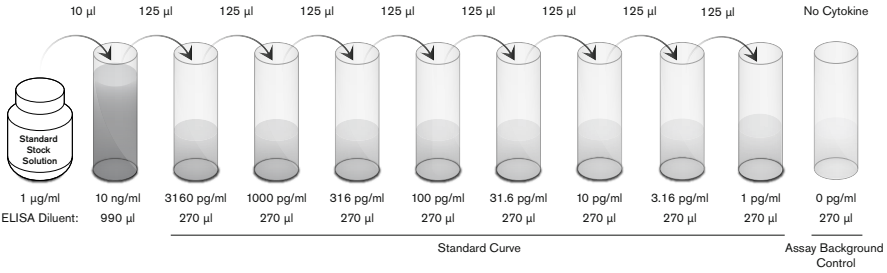

Figure 1. Recommended serial dilution of cytokine standard. The volumes indicated are sufficient for duplicates. The last vial should be 0 pg/ml of standard i.e. the analyte should be omitted.

Samples
Dilute all samples in "ELISA diluent" prior to use. We recommend the use of duplicate wells for all samples. It is recommended that visible precipitate in the sample should be removed.

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. For tube dilutions, equal volumes of "ELISA diluent" and sample should be mixed, e.g. 120 µl "ELISA diluent" + 120 µl sample for a duplicate. If sample dilutions are made directly in the plate, "ELISA diluent" (50 µl per well) should be added prior to addition of the sample (50 µl per well).

Detection antibody
Dilute the "Detection antibody" 2000x in "ELISA diluent" prior to use. For 1 plate, dilute 6 µl "Detection antibody" in 12 ml "ELISA diluent" which will give a final "Detection antibody" concentration of 0.5 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 2000x to a final concentration of 0.5 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. Note that cytokine values obtained should be multiplied with the dilution factor used for each sample.

PERFORMANCE OF THE ASSAY

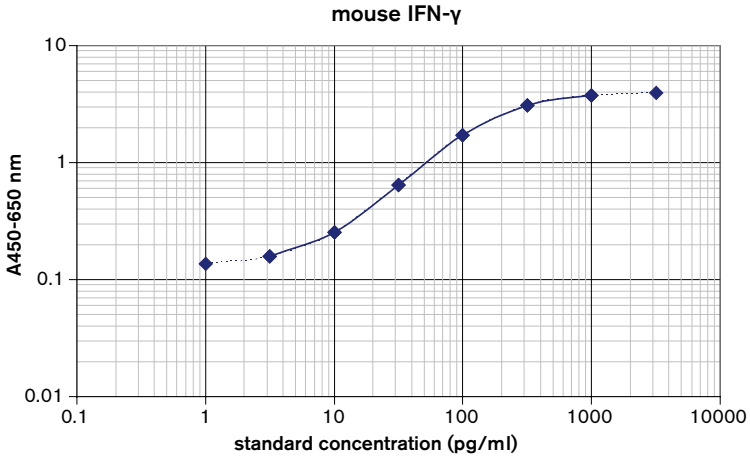

Figure 2. Display of the standard curve.

Standard range: 3.16-1000 pg/ml. The standard range is the range in which determinations of analyte concentration can be done with precision, accuracy and linearity.

Sensitivity: The limit of detection of this assay is 2 pg/ml. It is the lowest concentration that is possible to detect but not necessarily quantify with precision and accuracy.

Accuracy: The standard of this ELISA has been calibrated against an international standard from NIAID*. One ng of supplied standard equals 5 units of Gxg02-901-533 NIBSC-standard. Please note that the calibration is batch specific.

Spike recovery: Addition of a specified amount of standard to a serum/plasma sample gives a mid-curve recovery of 93-111% in repeated experiments.

Precision: The intraassay variation is 4.4%(CV). The interassay variation is 6.3%(CV).

*National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892-6612, USA.

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.

MABTECH AB
Augustendalstorget 9
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

MABTECH Inc
M.E.B. 220, 3814 West Street
Cincinnati, OH 45227
USA
Toll free: +1 866 ELI-SPOT
Tel: +1 513 871 4500
Fax: +1 513 871 7353
E-mail: mabtech.usa@mabtech.com

MABTECH Australia Pty Ltd
Australia
Tel: +61 3 9470 4704
Fax: +61 3 8678 3216
E-mail: mabtech.au@mabtech.com

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
Tel: +33 (0)4 92 38 80 70
Fax:+33 (0)4 92 38 80 71
E-mail: mabtech.fr@mabtech.com

MABTECH

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