Product datasheet

Note: This protocol was modified as of January 2015. All previous protocols are obsolete.

Mouse IFN-γ ELISA Kit

Product #: 0002

Storage recommendations

Store the kit at 2-8°C. The kit is stable for a period of up to 3 months from the date of receipt.

Description

This mouse IFN-γ ELISA kit is designed to enable the quantitative measurement of natural and/or recombinant IFN-γ in serum, plasma and cell culture media. The kit contains one strip-well plate pre-coated with mouse IFN-γ capture antibody along with sufficient key reagents for plate development.

Reagent preparation

Wash buffer (20x)

If crystals have formed in the concentrate, warm to room temperature and mix well. Once all the visible crystals have dissolved, dilute 25ml of wash buffer concentrate in 475ml of distilled water to make 500ml of wash buffer.

Standard

The standard is present in lyophilised form with the amount of standard present in the tube clearly stated on the label. Prior to reconstitution, briefly centrifuge the vial to ensure that any loose lyophilised material has collected at the bottom of the tube.

For cell culture samples:

Reconstitute the standard in 1mL of assay diluent 1B and mix well by inversion. **Allow the standard to dissolve for at least 15 minutes with regular inversion prior to use**. Dividing the amount of lyophilised standard (as per the label) by 1mL will give the standard concentration. **Use sample culture medium to perform the serial dilutions.**

For serum samples:

Reconstitute the standard in 1ml of assay diluent 1B and mix well by inversion. **Allow the standard to dissolve for at least 15 minutes with regular inversion prior to use**. Dividing the amount of lyophilised standard (as per the label) by 1mL will give the standard concentration. **Use standard diluent BS to perform the serial dilutions.**

For plasma samples:

Reconstitute the standard in 1ml of assay diluent 1B and mix well by inversion. **Allow the standard to dissolve for at least 15 minutes with regular inversion prior to use**. Dividing the amount of lyophilised standard (as per the label) by 1mL will give the standard concentration. **Use assay diluent 1B to perform the serial dilutions.**

The standard curve should be performed in duplicate starting at 1000pg/ml and diluted 1 in 2 down to at least 15.6pg/ml. Use the formula $C_1V_1=C_2V_2$ to calculate the amount of stock required to make 1ml of the 1000pg/ml standard. Perform this initial dilution in a micro tube ensuring that the appropriate diluent is used. Serial dilutions are then easily achieved by using a micro-titre plate (or similar). Load 300µL of the 1000pg/ml standard into the first 2 wells of the dilution plate and load 125µL of the appropriate diluent into the remaining wells. Using a multichannel pipette, transfer 125µL from the first wells (i.e. 1000pg/ml) into the second wells (now 500pg/ml), mix well by pipetting up and down before transferring 125µL to the next wells (now 250pg/ml). Repeat until the serial dilution is complete.

Detection antibody

Briefly centrifuge prior to use. The anti-mouse IFN-γ biotin labelled detection antibody is provided in a liquid form at the concentration specified on the tube. **The anti-mouse IFN-γ biotin labelled detection antibody should be diluted to 25ng/ml in assay diluent 1B immediately prior to use**.

Streptavidin-HRP conjugate

Briefly centrifuge prior to use. The streptavidin-HRP conjugate should be diluted 500 fold in assay diluent 1B immediately prior to use. For example, if developing the entire plate aliquot 22µL of streptavidin-HRP conjugate into 11ml of assay diluent 1B.

Protocol

It is highly recommended that all standards, samples and zero standard controls be performed in duplicate. Ensure that all reagents are at room temp prior to use.

1. Prepare solutions as required by following the instructions outlined in the reagent preparation section.

2. Remove any 8 well strips that are not required for the assay and place back in the foil pouch ensuring that it is closed tightly

3. Add 100µL of standards, samples and zero standard controls to each well and seal the plate with the adhesive cover provided. Incubate for 2 hours at room temperature.

4. Aspirate each well and wash the plate 4x with wash buffer (250µL). Washing can be achieved with a multichannel pipette, plate washer or similar. To remove residual wash buffer invert the plate and tap the plate on paper towels between each wash.

5. Add 100µL of biotin labelled detection antibody to each well and seal the plate with the adhesive cover provided. Incubate for 1 hour at room temperature.

6. Repeat the aspiration/wash steps in step 4.

7. Add 100µL of freshly diluted streptavidin-HRP conjugate to each well and seal the plate with the adhesive cover provided. Incubate for 45 minutes at room temperature.

8. Aspirate each well and wash the plate **5x** with wash buffer (250µL) as per step 5. It is important to wash thoroughly here to reduce unwanted background.

9. Add 100µL of TMB substrate to each well and incubate for approximately 15 minutes at room temperature. **Ensure the plate is protected from light**. Check the development process (blue colour) every 5 minutes or so to prevent over development of the plate.

10. Stop the reaction with 50µL of stop solution. The wells will turn from blue to yellow in colour.

11. Determine the optical density (OD) of the plate using a micro-plate reader set at 450nm. If the micro-plate reader allows wavelength correction, set at 570nm.

Calculation of results

Calculate the mean absorbance for each standard, sample and control and subtract the mean of the zero standard controls.

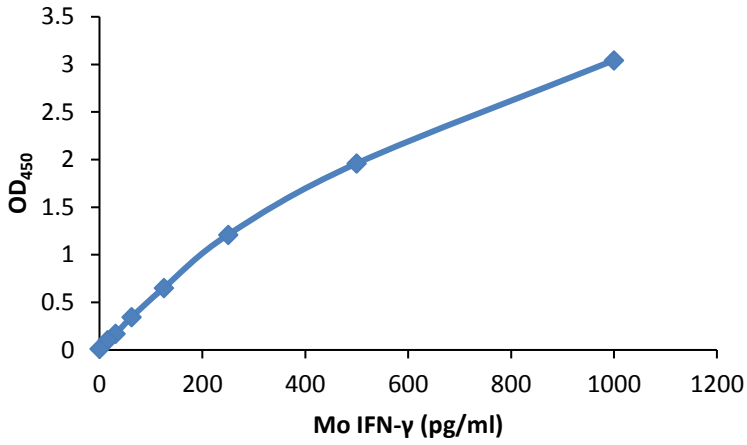
Create a standard curve by plotting the mean OD of each standard on the y-axis versus the standard concentration on the x-axis. If available, use a program capable of generating a four parameter logistic (4-PL) curve fit. A free user friendly 4-PL program is available online at http://elisaanalysis.com. Alternatively, plot a best fit curve through the standard points and overlay a linear or polynomial trend-line. The resultant equation displayed on the graph can then be used to

calculate the unknown concentrations by solving for x. When fitting a trend-line it is essential that the r^2 value is 0.99 or higher. If not, then simply find the linear portion of the graph by removing the higher standard points from the analysis.

If the samples were diluted then multiply the concentration read from the standard curve by the dilution factor.

Standard curve

This is a representative example of a typical standard curve. A separate standard curve must be generated for each experiment.



Sensitivity

The lower limit of quantitation (LLQ) of the assay is typically <5pg/ml.

The LLQ was determined by adding 3 standard deviations to the mean optical density of 5 zero antigen replicates and calculating the corresponding concentration.

Precision.

Intra-assay CV: <10%

Inter-assay CV: <10%

Recovery.

Various matrix types were spiked with recombinant mouse IFN-γ and compared to a spiked assay diluent control. Typical values are as follows.

Sample	100pg/ml	50pg/ml	25pg/ml
Cell culture media	108	99	102
Serum	102	103	114

Plasma	88	88	93

Linearity. Linearity of dilution was determined by serially diluting spiked samples and analysing them in the ELISA.

Sample	Dilution factor	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
Cell culture media	neat	114	-	-
	1:2	57	49	85
	1:4	29	25	86
Serum	neat	108	-	-
	1:2	54	51	94
	1:4	27	28	103
Plasma	neat	94	-	-
	1:2	43	43	98
	1:4	23	22.5	103

Cross reactivity

This ELISA kit is specific for natural and recombinant mouse IFN-γ. It does not cross react with IL-1α, IL-1β, IL-3, IL-4, IL-6, IL-7, IL-10, GM-CSF, TNF-α or human IFN-α or IFN-γ.

Technical hints

Always ensure complete reconstitution and/or dilution of reagents prior to use.

To ensure uniform temperature/humidity levels for all steps of the ELISA across the entire plate it is often beneficial to perform the incubations in a humidity chamber. This can simply be a Styrofoam box containing moistened paper hand towels.

Plates should always be incubated with the adhesive plate sealer in place.

Always ensure accurate pipetting of reagents at all times, especially the standards.

Prior to reading the plate OD, ensure that the underside of the plate is clean. If not, clean with a lint free wipe.