MABTECH

ELISA Flex: Mouse IFN-α (HRP)

3326-1H-6 | 3326-1H-20

ELISA Flex kit for quantitative determination of native and recombinant mouse IFN-α in solution, e.g. cell supernatant and serum/plasma.

The kit includes		3326-1H-6 for 6 plates	3326-1H-20 for 20 plates
Capture mAbs:	MT24A/44A/104 (0.5 mg/ml)	350 µl	1200 µl
Detection mAbs:	MT9L/14A/113, biotin (0.5 mg/ml)	150 µl	500 µl
Streptavidin-HRP		80 µl	250 µl
Recombinant mouse IFN-α ELISA standard		1 vial	1 vial
Standard reconstitution buffer A5		1 ml	1 ml

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

Shipping and storage

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. Antibodies are supplied in sterile-filtered PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 0.002% Kathon CG. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

General and Preparations

Specificity

The kit contains matched monoclonal antibodies (mAbs) specific for native and recombinant mouse IFN-α. Subtypes 1, 2, 4, 5, 6T, 7/10 (129/Sv), 8/6, 9, 11, 12, 13, 14, 15 (A), and B are detected.

Standard range

8-1000 pg/ml

Calibration

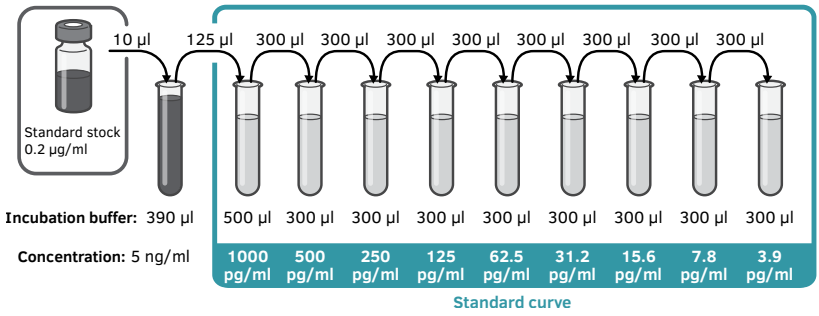
No international standard exists for calibration.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.2 µg/ml by adding 1 ml of the 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

Prepare within 30 minutes of use. Volumes are sufficient for duplicates.



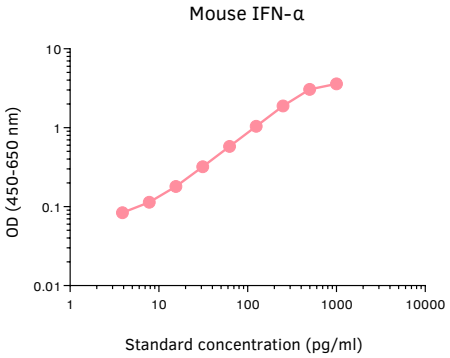
Protocol

Day 1

1. Add 100 µl/well of capture mAbs MT24A/44A/104 diluted to 3 µg/ml in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 °C.

Day 2

2. Empty the plate and add 200 µl/well of PBS with 0.05% Tween 20 and 0.1% BSA (incubation buffer) to block the plate. Incubate for 1 hour at room temperature.
3. Wash the plate 5 times with PBS containing 0.05% Tween 20 (300 µl/well).
4. Add 100 µl/well of samples or standards diluted in incubation buffer. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
5. Wash as above.
6. Add 100 µl/well of detection mAb MT9L/14A/113-biotin diluted to 1 µg/ml in incubation buffer. Incubate for 1 hour at room temperature.
7. Wash as above.
8. Add 100 µl/well of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. Please note that sodium azide used in buffers will inhibit HRP activity.
9. Wash as above.
10. Add 100 µl/well of TMB substrate (product code: 3652-F10) and incubate at room temperature, protected from direct light for 15 minutes.
11. Add 100 µl/well of 0.2 M H_2SO_4 to stop the reaction.
12. Measure the optical density in an ELISA reader at 450 nm within 15 min. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.



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Mabtech AB (Head Office)
Sweden
Tel: +46 8 716 27 00
mabtech@mabtech.com

Mabtech, Inc.
USA
Tel: +1 513 871-4500
mabtech.usa@mabtech.com

www.mabtech.com