

# ELISA Flex: Human IFN- $\gamma$ (ALP)

3420-1A-6 | 3420-1A-20

ELISA Flex kit for quantitative determination of native and recombinant human IFN- $\gamma$  in solution, e.g. cell supernatant and serum/plasma.

<b>The kit includes</b>		<b>3420-1A-6</b> for 6 plates	<b>3420-1A-20</b> for 20 plates
Capture mAb:	1-D1K (1 mg/ml)	150 $\mu$ l	500 $\mu$ l
Detection mAb:	7-B6-1, biotin (1 mg/ml)	80 $\mu$ l	250 $\mu$ l
Streptavidin-ALP		80 $\mu$ l	250 $\mu$ l
Recombinant human IFN- $\gamma$ ELISA standard		1 vial	1 vial
Standard reconstitution buffer A8		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. Antibodies are supplied in sterile-filtered PBS with sodium azide (0.02%). Streptavidin-ALP is supplied in 0.1 M Tris buffer 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 a matched pair of monoclonal antibodies (mAbs) specific for native and recombinant human IFN- $\gamma$ .

## Standard range

4-400 pg/ml

## Calibration

The ELISA standard has been calibrated against an international standard from the National Institute of Allergy and Infectious Diseases (NIAID) Bethesda, US. One ng of supplied standard equals 39 U Gxg01-902-535 NIAID-standard. Please note that the calibration is batch specific.

## Analysis of serum and plasma samples

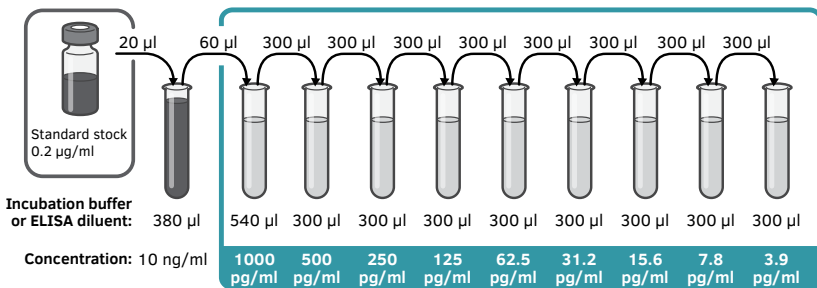
Analysis of serum/plasma requires the use of ELISA diluent (product code: 3652-D2). The ELISA diluent blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The ELISA diluent should be used for dilution of standard, samples, and detection antibody.

## Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.2  $\mu\text{g/ml}$  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  $-20\text{ }^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

## Preparation of standard curve

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



Standard curve

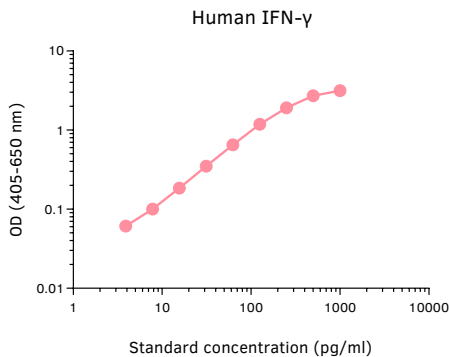
# Protocol

## Day 1

1. Add 100  $\mu\text{l}$ /well of capture mAb 1-D1K diluted to 2  $\mu\text{g}/\text{ml}$  in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8  $^{\circ}\text{C}$ .

## Day 2

2. Empty the plate and add 200  $\mu\text{l}$ /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  $\mu\text{l}$ /well).
4. Add 100  $\mu\text{l}$ /well of samples or standards diluted in incubation buffer or ELISA diluent. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
5. Wash as above.
6. Add 100  $\mu\text{l}$ /well of detection mAb 7-B6-1-biotin diluted to 1  $\mu\text{g}/\text{ml}$  in incubation buffer or ELISA diluent. Incubate for 1 hour at room temperature.
7. Wash as above.
8. Add 100  $\mu\text{l}$ /well of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
9. Wash as above.
10. Add 100  $\mu\text{l}$ /well of pNPP substrate (product code: 3652-P10) and incubate at room temperature protected from direct light for approximately 60 minutes.
11. Measure the optical density in an ELISA reader at 405 nm. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.



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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**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