## Datasheet & Protocol

## MABTECH

# ELISA Flex: Llama IFN-γ (ALP)

3123-1A-6 | 3123-1A-20

ELISA Flex kit for quantitative determination of llama IFN- $\gamma$  and alpaca IFN- $\gamma$  in solution, e.g. cell supernatant.

The kit includes	<b>3123-1A-6</b> for 6 plates	<b>3123-1A-20</b> for 20 plates
Capture mAb: bIFNγ-I (0.5 mg/ml)	300 μl	1000 μl
Detection mAb: PAN, biotin (0.5 mg/ml)	40 µl	120 µl
Streptavidin-ALP	80 µl	250 µl
Recombinant llama IFN-γ 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, except the standard which should be stored at -20 °C. 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 bovine IFN- $\gamma$ . The mAbs cross-react with IFN- $\gamma$  from llama, alpaca, sheep, horse, and dog.

Standard range 7-700 pg/ml

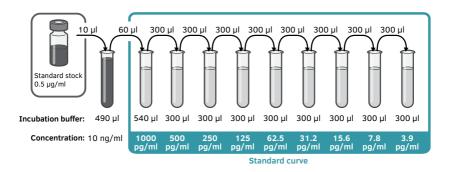
**Calibration** No international standard exists for calibration.

#### **Reconstitution of ELISA standard**

Reconstitute the ELISA standard to a stock solution of 0.5  $\mu$ 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 °C. Avoid repeated freeze-thaw cycles.

#### Preparation of standard curve

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



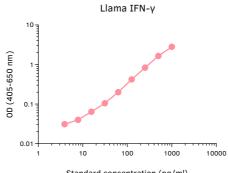
### Protocol

Dav 1

1. Add 100 µl/well of capture mAb bIFNy-I diluted to 2 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  $\mu$ 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 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 PAN-biotin diluted to 0.2 μg/ml in incubation buffer. Incubate for 1 hour at room temperature.
- 7. Wash as above.
- 8. Add 100 µl/well of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
- 9. Wash as above.
- **10.** Add 100 µl/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.



Standard concentration (pg/ml)



Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.



#### The products are for research use only.

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