# SIOLOGICALS a biotechne brand

## ELISA PRODUCT INFORMATION & MANUAL

## IFN-gamma ELISA Development Kit NBP3-11746

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only. Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

#### **Datasheet & Protocol**

### IFN-GAMMA ELISA DEVELOPMENT KIT

The kit includes		NBP3-11746 for 6 plates	
Capture mAb:	MTF14 (0.5 mg/ml)	300 µl	
Detection mAb:	MTF19, biotinylated (0.5 mg/ml)	150 µl	
Streptavidin-HRP		80 µl	
Recombinant ferret IFN-y ELISA standard		1 vial	
Standard reconstitution buffer A8		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 a matched pair of monoclonal antibodies (mAbs) specific for native and recombinant ferret IFN- $\gamma.$ 

Standard range

12-1200 pg/ml

Calibration

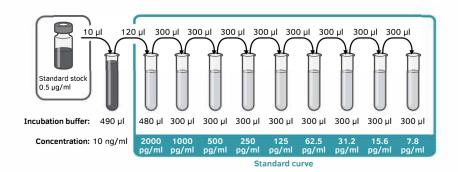
No international standard exists for calibration.

#### **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.

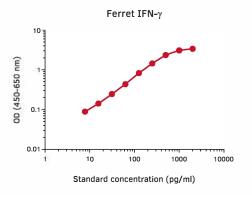
**Preparation of standard curve** 

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



#### Protocol

- **Day 1 1.** Add 100 μl/well of capture mAb MTF14 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 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  $\mu$ l/well).
  - 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.
  - Add 100 µl/well of detection mAb MTF19-biotin diluted to 1 µg/ml in incubation buffer. Incubate for 1 hour at room temperature.
  - 7. Wash as above.
  - 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 and incubate for 15 minutes.
  - **11**. Add 100  $\mu$ l/well of 0.2 M H<sub>2</sub>SO<sub>4</sub> 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.



Quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.

The products are for research use only.

