

# ELISA PRODUCT INFORMATION & MANUAL

### IFN-gamma ELISA Development Kit NBP3-11750

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.

Not for diagnostic or therapeutic procedures.

## IFN-GAMMA ELISA DEVELOPMENT KIT

ELISA Development Kit for quantitative determination of native and recombinant chicken IFN-y in solution, e.g. cell supernatant.

The kit includes	NBP3-11750 for 6 plates
Capture mAb: MT6C2 (0.5 mg/ml)	300 µl
Detection mAb: MT7C10, biotinylated (0.5 mg/ml)	150 µl
Streptavidin-HRP	80 μΙ
Recombinant chicken IFN-γ 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  $^{\circ}$ C upon receipt, except the standard which should be stored at -20  $^{\circ}$ 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 chicken IFN-y.

#### Standard range

5-1000 pg/ml

#### Calibration

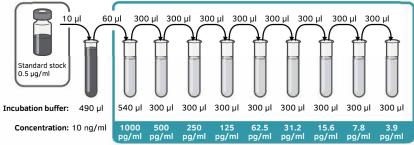
No international standard exists for calibration.

#### **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. Use immediately or store at +4°C for maximum 6 months.

#### **Preparation of standard curve**

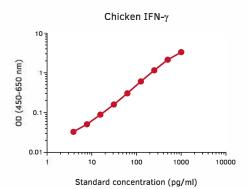
Prepare within 30 minutes of use. Volumes are sufficient for duplicates.



Standard curve

#### **Protocol**

- Day 1 1. Add 100 μl/well of capture mAb MT6C2 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 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 MT7C10-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 and incubate for 15 minutes.
  - 11. Add 100  $\mu$ I/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.

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