ELISA PRODUCT INFORMATION & MANUAL

IFN-gamma ELISA Development Kit

NBP3-11749

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

IFN-GAMMA ELISA DEVELOPMENT KIT

ELISA Development Kit for quantitative determination of llama IFN-gamma and alpaca IFN-gamma in solution, e.g. cell supernatant.

The kit includes		NBP3-11749 for 6 plates
Capture mAb:	bIFNγ-I (0.5 mg/ml)	300 µl
Detection mAb:	PAN, biotinylated (0.5 mg/ml)	40 µl
Streptavidin-HRP		80 µl
Recombinant llama 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 °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 bovine IFN-gamma. The mAbs cross-react with IFN-gamma from llama, alpaca, sheep, horse, and dog.

Standard range

7-700 pg/ml

Calibration

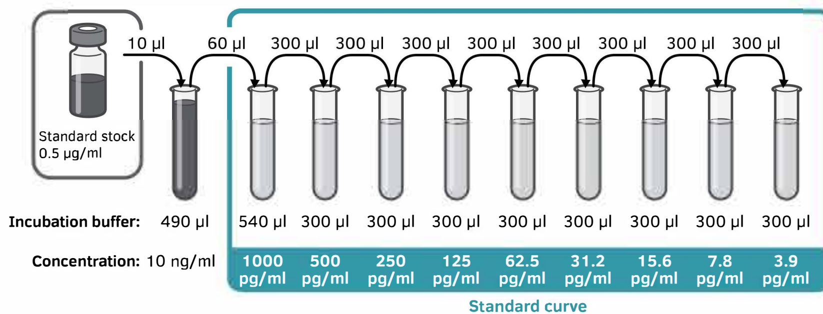
No international standard exists for calibration.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.5 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.



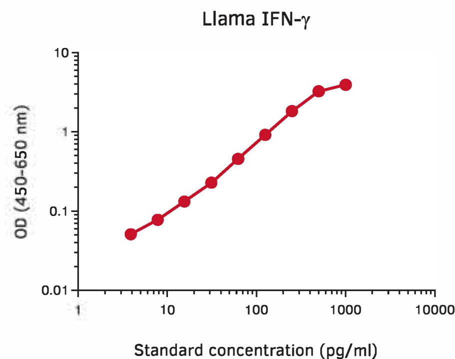
Protocol

Day 1

1. Add 100 µl/well of capture mAb bIFNγ-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 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 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-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 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.



Updated on 2022/02/23

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

The products are for research use only.