# Datasheet & Protocol



# ELISA Flex: Cotton rat IFN-γ (ALP)

3121-1A-6 | 3121-1A-20

ELISA Flex kit for quantitative determination of native and recombinant cotton rat IFN-y in solution, e.g. cell supernatant and serum/plasma samples.

The kit includes		<b>3121-1A-6</b> for 6 plates	<b>3121-1A-20</b> for 20 plates
Capture mAb:	MT413 (0.5 mg/ml)	300 μΙ	1000 μΙ
Detection mAb:	MT417, biotin (0.5 mg/ml)	150 μΙ	500 μΙ
Streptavidin-ALP		80 μΙ	250 μΙ
Recombinant Cotton rat 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 native and recombinant cotton rat IFN-y.

#### Standard range

6-600 pg/ml

#### Calibration

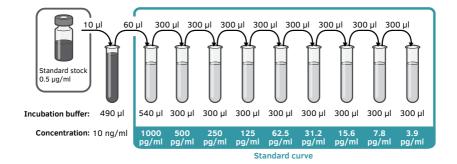
No international standard exists for calibration.

#### 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.



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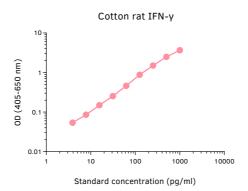
# **Protocol**

#### Day 1

1. Add 100  $\mu$ l/well of capture mAb MT413 diluted to 2  $\mu$ g/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 MT417-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-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.



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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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