MONOSCREEN Quant ELISA

Instruction manual
**BIOK093-IFNG_NO_(EN)_V05**
**25/02/2026**

# Monoscreen QuantELISA Gamma Interferon

Reference : BIO K 093

ELISA kit for the semi-quantitative dosage of Gamma Interferon for bovine

Monowell, sandwich test

For veterinary *in vitro* use only

| Sample | Species | Individual analysis |
|---|---|---|
| Plasma | Bovine (ovine, caprine) | ✓ |

## Presentation

| Product reference | BIO K 093/2 | BIO K 093/30 |
|---|---|---|
| Format | 2 plates, strips of 8 wells | 30 plates, strips of 8 wells |
| Reactions | 192 wells | 2880 wells |

## Kit composition

| Provided material | | Type* | Code | BIO K 093/2 | Code | BIO K 093/30 |
|---|---|---|---|---|---|---|
| Microplate | Microplate | 1 | D00559 | 2 | D00559 | 30 |
| Plate covers | Plate covers | - | A01301 | 1 | A01301 | 6 |
| Washing solution (20X) | Washing solution (20X) | A | D00695 | 1 x 100 mL | D00696 | 3 x 250 mL |
| Dilution solution (1X) | Colored dilution solution (1X) | B | D01612 | 1 x 125 mL | D01613 | 2 x 250 mL |
| TMB solution (1X) | Single component TMB (1X) | A | D01585 | 1 x 30 mL | D01530 | 2 x 200 mL |
| Stop solution (1X) | Stopping solution (1X) | A | D00680 | 1 x 30 mL | D01531 | 2 x 200 mL |
| Conjugate (50X) | Conjugate (50X) | 1 | D01611 | 1 x 0,6 mL | D01528 | 1 x 10 mL |
| CTL POS | Positive control (freeze-dried) | 1 | D01562 | 1 | D01562 | 3 |
| CTL NEG | Negative control (freeze-dried) | a | D01593 | 1 | D01593 | 3 |
| IFU | Instructions for use | - | - | 1 | - | 1 |
| CoA | Quality control certificate | - | - | 1 | - | 1 |
| Information note | Information note | - | - | 1 | - | 1 |

*: (1): dependent on kit and batch : (a): dependent on kit / (A): substitutable with components A / (B): substitutable with components B.

## Revision history

| Date | Version | Modifications |
|---|---|---|
| 16/12/2024 | V01 | Initial version reviewed and approved by competent authorities |
| 25/11/2025 | V02 | Modification of positive and negative controls in lyophilized format |
| 16/12/2025 | V03 | Adjustment of component interchangeability and addition of the information note. |
| 19/02/2026 | V04 | Change in the number of bottles of washing solution.<br>Change in the interpretation threshold for the PBS ratio. |
| 25/02/2026 | V05 | Adjustment of components volume. |

Note : minor typographical, grammar and formatting changes are not included in the revision history.

## A. Introduction

Interferon gamma (IFN-γ) is secreted mainly by activated T lymphocytes and Natural Killer (NK) cells whose synthesis is stimulated by Interleukin-18 (IL-18). IFN-γ is involved in the regulation of immune and inflammatory responses.

## B. Test principle

The test uses 96-well microtitration plates sensitized with a specific monoclonal antibody for the bovine gamma Interferon.

In each well, 50 µL of plasma is distributed. After a 60-minute incubation and a rinse step, the operator adds the conjugate, which is a monoclonal antibody specific for bovine gamma interferon coupled with peroxidase. After this incubation, the plate is washed, and the operator adds the chromogen tetramethylbenzidine.

This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and of not being carcinogenic. The optical density reading is done at 450 nm. The intensity of the color is proportionate to the titre of specific antibody in the sample. Positive and negative controls are added to the kit to allow the validation of the test results.

## C. Material required but not provided

- Distilled/demineralized water.
- Graduated electronic mono or multichannel pipettes (2-20µL, 20-200µL and 10-1000µL range) and single-use tips.
- Plate agitator.
- Microplate reader (450 nm filter).
- Microplate washer.
- Incubator at 22±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,…

## D. Warnings and precautions of use

- The reagents must be kept at 5±3°C.
- Store the kit controls at a temperature ≤-16°C.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminum envelope.
- Do not use reagents beyond shelf-life date.
- Do not use reagents from other batches.
- Make sure to use distilled/demineralized water.
- The stopping solution contains 1M phosphoric acid. Handle it carefully.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- Keep the TMB solution away from light.

## E. Preparation of the solutions

- **The solutions are to be prepared extemporaneously.**
- The washing solution must be diluted 20-fold in distilled/demineralized water. The cold solution crystallizes spontaneously. Dissolve the crystals by keeping the solution at room temperature or bring it at 37±2°C in an oven or a bath water ; mix the solution well and withdraw the necessary volume.
- The dilution solution is ready to use. The dilution is colored in yellow. It is used for dilution of conjugate.
- The conjugate must be diluted 50-fold in the dilution solution (it must not be vortexed, or frozen).
- The stopping solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.
- The positive and negative controls are lyophilized. They should be rehydrated with distilled water, refer to the information note. For optimal storage, make aliquots and freeze them at a temperature ≤-20°C, avoiding freeze/thaw cycles.

## F. Samples

*The plasmas analyzed by this method are derived from blood collected on lithium heparin, then stimulated within eight hours of collecting the animal's blood sample with mycobacteria-specific antigens (PPDa / PPDb, Mix PC-EC), a Pokeweed mitogen (PWM) for immunocompetent control of cell viability and a blank control (PBS) to verify the absence of basal IFNγ production face to another type of active infection at the time of blood sample).*

This sample collection and stimulation step, which is approved by the competent health authority, is outside the scope of this assay method and must be carried out in accordance with the current version of the official ANSES/LSAn/UZB-LNR Tub/M1 method.

- **Storage of collected plasmas**

Plasma samples recovered after this stimulation step can be stored at 5±3°C ideally for 2-3 days if they are not tested on the day of collection. For longer storage, samples should be kept frozen at a temperature ≤-20°C.

## G. Procedure

1. Bring all the reagents to room temperature before use.
2. Distribute duplicate controls and plasmas to be tested at a rate of 50 µL per well.
3. Cover each microplate with a lid (in case of manual method) and incubate at 22±3°C for 60±5 min.
4. Using a washer, or manually, perform 3 successive washes with 300 µL of wash solution per well.

   N.B: Avoid the formation of bubbles in the wells. Do not allow microplates to dry between washes. Tap the microplates on clean absorbent paper after the final wash step to remove any remaining wash solution.
5. Add 100 µL per well of freshly diluted conjugate. Cover with a lid (in case of manual method) and incubate plates at 22±3°C for 60±5 min.
6. Wash plates as in the previous step (see §4).
7. Dispense 100 µL of TMB solution per well.

   NB: Do not treat more than 6 plates at a time, so as to respect the 10±1 min of the next step for all wells.
8. Incubate at 22±3°C for 10±1 min. in the dark, **without covering the plates**.
9. Dispense 100 µL of stop solution per well. Color should change from blue to yellow.
10. Within 5 minutes of adding the stop solution, measure optical densities (OD) using a microplate spectrophotometer with a 450 nm filter.

## H. Validation of results

### 1. Plate validation criteria

Controls results must be examined before the samples' results may be interpreted.

Calculate the mean absorbance (DO) of negative controls (NC) and positive controls (PC). The test is **valid**, only if:

- The mean OD of the IFN-γ negative control was <0.130, with a range between different NC values ≤0.040.
- The mean OD of the IFN-γ positive control is >0.700, with a difference between different PC values on the same plate ≤20%.

If any of the above criteria are not met, the ELISA is not validated and must be repeated.

### 2. Samples results analysis

Calculate for each sample:

The PPD ratio: PPD Sample = [(PPDb – PPDa) / (PC – NC)] x 100

The PPDb ratio: PPDb Sample = [(PPDb – PBS) / (PC – NC)] x 100

The MIX ratio: MIX Sample = [(MIX – PBS) / (PC – NC)] x 100

The PWM ratio: PWM Sample = [(PWM – PBS) / (PC – NC)] x 100

The PBD ratio: PBS Sample = [PBS / (PC – NC)] x 100

## I. Interpretation of results

Results are validated or uninterpretable depending on the values of the PWM ratio and PBS ratio thresholds.

| PWM Ratio | PWM interpretation |
|---|---|
| PWM > 30% | Validated |
| PWM ≤ 30% | Uninterpretable |

| PBS ratio | PBS interpretation |
|---|---|
| PBS < 60% | Validated |
| PBS ≥ 60% | Uninterpretable |

| Sample | PPD ratio | Mix ratio | PPDb ratio |
|---|---|---|---|
| Negative | PPD < 5% | MIX < 3% | PPDb ≤ 70% |
| Positive | PPD ≥ 5% | MIX ≥ 3% | PPDb > 70% |

**The values of these thresholds and ratios are defined by the competent authority and are subject to change: please refer to the current State Service Note.**

***Caution: Immunosuppression caused by recent treatment with dexamethasone treatment or calving may depress IFN-γ responses to mycobacterial antigens. Animals that have received a dexamethasone injection within a week or calved within 4 weeks should be retested to reduce the possibility of a false-negative result.***

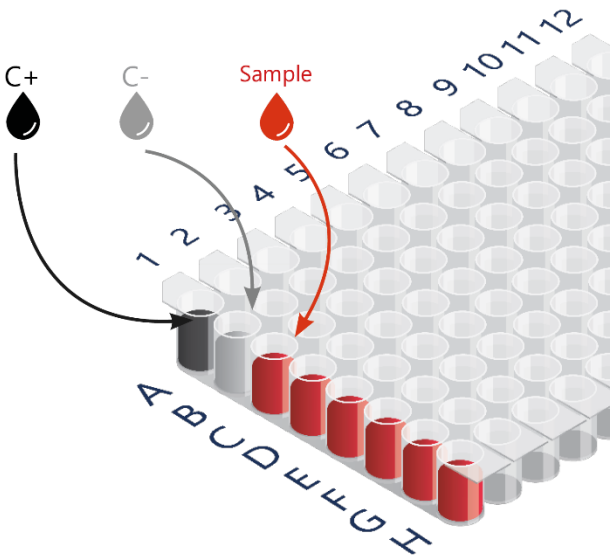
## Symbols

| Symbol | Meaning |
|---|---|
|  | Catalog number |
|  | Manufacturer |
|  | Temperature limit |
|  | Use by |
|  | Batch code |
|  | Consult Instructions for Use |
|  | Contain sufficient for "n" tests |
|  | Keep away from light |
|  | Keep dry |
|  | Corrosive substance |
|  | Hazardous/irritating product |

## Notes*

**1** Distribute 50µL of samples and rehydrated kit controls (positive and negative control)

Room Temp. — 60 min → 3X Wash



*Kit microplate*

**2** Add 100 µL of diluted conjugate (1/50)

Room Temp. — 60 min → 3X Wash

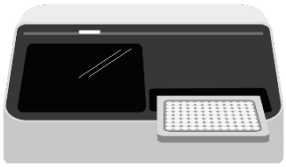
**3** Add 100 µL of TMB

Room Temp. — 10 min — Obscurity

**4** Add 100 µL of stop solution

**5** Record optical densities

450 nm



* Notes do not replace the instructions of use of which they are a summary.



Contact us
support.immuno@biox.com
www.biox.com +32 (0) 84 32 23 77
Bio-X Diagnostics
38, rue de la Calestienne
5580 Rochefort (Belgium)