General Information

Interferon gamma (IFN-g) is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by CD4 and CD8 cytotoxic T lymphocyte (CTL) effector cells once antigen-specific immunity develops.

This sandwich ELISA is designed to detect native bovine, caprine and ovine interferon gamma (IFN-g) in plasma or culture supernatant. It uses two different monoclonal antibodies against ruminant IFN-g, and incorporates native ruminant IFN-g as a positive control/standard. Results are expressed with respect to a standardized, freeze-dried positive reference control. This relative expression of the measured quantity of IFN-g guarantees the standardization of results between runs and kit batches.

Note 1: Recombinant interferon can also be detected. However, the degree of recognition of recombinants can vary between techniques, and may also depend on the dilution buffer used.

Note 2: A protocol for quantitative IFN-g determination can be provided upon request.

Description and Principle

Wells are coated with an anti-IFN-g monoclonal antibody (Mab).

Samples to be tested and controls are added to the microwells. IFN-g, if present, forms a Mab-antigen complex. After washing, an anti-IFN-g Mab–HRP conjugate is added, forming a Mab-antigen-Mab-HRP complex. After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added.

The resulting coloration depends on the quantity of IFN- γ present in the sample to be tested:

- in the presence of IFN-g, a blue solution appears which becomes yellow after addition of the stop solution.
- in the absence of IFN-g, no coloration appears.

The microplate is read at 450nm.

Kit Components

Reagents*
Microplates coated with anti-ruminant IFN-g Mab
Anti- ruminant IFN-g concentrated HRP conjugate (10X)
Positive Control, freeze-dried.
Positive Control reconstitution solvent
Negative Control
Dilution Buffer 1
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

** Quantities supplied are indicated on the kit label.*

1. The dilution buffer 1, the conjugate, the controls and the substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. Components bearing the same name (*wash solution, dilution buffers*) can be used for the entire IDvet product range.

Materials required but not provided

1. 37°C incubator
2. Mono or multi-channel micropipettors capable of delivering volumes of 5µl*, 10µl, 100µl, and 200µl.
3. Disposable tips.
4. 96-well microplate reader.
5. Distilled or deionized water.
6. Manual or automatic wash system.

* Required precision = 5µl ± 1µl

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0.5 M) may be harmful if swallowed. It may cause sensitisation by skin contact (**R22-43**). Avoid contact with skin (**S24-37**).
4. Do not expose the substrate solution to bright light nor to oxidizing agents.
5. Decontaminate all reagents before elimination

Sample Preparation

In order to avoid differences in incubation times between samples, it is possible to prepare a 96-well plate containing the test and control samples, before transferring them into an ELISA microplate using a multi-channel pipette.

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature (21°C ± 5°C) and mix thoroughly to ensure that the Wash Concentrate is completely solubilized.

Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20X)** in distilled / deionized water.

Reagent preparation and storage

Allow all reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or Vortex.

Positive control:

Reconstitute freeze-dried **positive control** with the **Positive Control Reconstitution Solvent** supplied in the kit. Volume to be added is mentioned on the label of each vial. Wait approximately 5 minutes, and mix gently but thoroughly. Ensure complete resolubilisation.

Once re-suspended, the **Positive Control** can be stored:

- **for 1 week at 5°C (± 3°C)** ;
- for longer term storage, **aliquot and freeze** (<-20°C). Each aliquot can support **3 freezing-thawing cycles** without loss of activity and can be stored until the kit expiry date.

Testing Procedure

Specific cellular activation:

For determination of T-cell antigen-specific response, each sample must be incubated in parallel using sample activated with the specific antigen (eg. PPD_{bovis}, **activated sample**) and a control antigen (eg. PPD_{avium}, **control sample**) and / or PBS.

Upon request, IDvet will provide a technical bulletin concerning sample collection, storage and activation.

Protocols:

Two protocols may be used for this test.

Protocol 1 is designed for regions where there is a high frequency of animals which react strongly to PPD_{avium}. These samples produce very high OD levels when tested for Bovine Tuberculosis. In order to bring these OD levels into the linear region of OD measurement, samples are strongly diluted (1:10) in Protocol 1.

Protocol 2 may be used for regions without a strong PPD_{avium} response; for zones with a weak response to mitogen; or when using recombinant antigens or peptides.

It is suggested that laboratories first test a selection of samples using Protocols 1 and 2. If the OD levels for the PPD_{avium} samples remain low, then Protocol 2 should be used for routine testing.

Protocol 1: For regions with a strong PPD_{avium} response:

Add:

- 25 µl of **Dilution Buffer 1** and 25 µl of the **Negative Control** to wells A1 and B1.
- 25 µl of **Dilution Buffer 1** and 25 µl of the **Positive Control** to wells C1 and D1.
- 90 µl of **Dilution Buffer 1** and 10 µl of each sample to be tested (**activated and control samples**) to the remaining wells.

Note: If you require additional Dilution buffer 1 for protocol 1, please contact info@id-vet.com.

Protocol 2: For regions without a strong PPD_{avium} response; for zones with a weak response to mitogen; for the use of recombinant antigens or peptides:

Add:

-25 µl of **Dilution Buffer 1** to each well.

-25 µl of the **Negative Control** to wells A1 and B1.

-25 µl of the **Positive Control** to wells C1 and D1.

-25 µl of each sample to be tested (**activated and control samples**) to the remaining wells.

For both protocols:

2. Agitate the plate for 2 min (± 1 min) at 21°C± 5°C).
3. Cover the plate with a plastic sheet cover and incubate **1 hour** ± **5 min** at 37°C (± 2°C).
4. Wash each well **6** times with approximately 300 µl of the **Wash Solution**. Avoid drying of the wells between washings.
5. Prepare the **Conjugate 1X** by diluting the **Conjugate 10X** to 1/10 in **Dilution Buffer 1**
6. Add 100 µl of the **Conjugate 1X** to each well.
7. Cover the plate with a plastic cover or a foil and incubate **1 hour** ± **5 min** at 37°C (± 2°C).
8. Wash each well **6** times with approximately 300 µl of the **Wash Solution**. Avoid drying of the wells between washings.
9. Add 100 µl of the **Substrate Solution** to each well.
10. Incubate **15 min** ± **2 min** at 21°C (± 5°C) in the dark.
11. Add 100 µl of the **Stop Solution** to each well in order to stop the reaction.
12. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean value of the Positive Control O.D. (OD_{PC}) is greater than 0.500.

$OD_{PC} > 0.500$

✓ the ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is greater than 3.

$OD_{PC} / OD_{NC} > 3$

Interpretation

For each sample, calculate the S/P, expressing the level of interferon production in percentage of the Positive Control as follows:

$$S/P\% = \frac{OD_{\text{activated sample (ex. PPDbovis)}} - OD_{\text{control sample (ex. PPDavium)}}}{OD_{PC} - OD_{NC}} \times 100$$

Protocol 1:

Samples with an S/P %:

- less than 15 % are considered negative (no IFN-g production induced by the antigen tested);
- greater than or equal to 15 % are considered positive (specific IFN-g production induced by the antigen tested)

Result	IFN-g production	STATUS
S/P % < 15%	NO	NEGATIVE
S/P % ≥ 15 %	YES	POSITIVE

Protocol 2:

Samples with an S/P %:

- less than 35 % are considered negative (no IFN-g production induced by the antigen tested)
- greater than or equal to 35 % are considered positive (specific IFN-g production induced by the antigen tested)

Result	IFN-g production	STATUS
S/P % < 35%	NO	NEGATIVE
S/P % ≥ 35 %	YES	POSITIVE

Note: Special caution should be taken when interpreting samples for which the OD values for PPD_{avium} and PPD_{bovis} are greater than 2.5

$OD_{\text{Non-specific Ag (PPDavium)}} > 2.5$ and $OD_{\text{Specific Ag (PPDbovis)}} > 2.5$

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Innovative Diagnostics

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ID Screen®
Ruminant IFN-g

Kit for the detection of bovine, ovine and caprine interferon gamma (IFN-g) by sandwich ELISA

For *in vitro* use

December 2012:
- Advice on which protocol to use is indicated in the Testing Procedure
- The cut-off is modified

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IDvet, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.id-vet.com - E-mail: info@id-vet.com