Kit contents

cattletype IFN-gamma	**(1)**
Cat. no.	**CT270401**
Number of plates	**1**
Test Plate: microtiter plate with 96 wells, coated with anti-ruminant IFN-γ monoclonal antibody	1
Sample Diluent, ready to use	1 x 15 ml
Negative Control, ready to use	1 x 1 ml
Positive Control	see label
Wash Buffer, 20x concentrate	1 x 60 ml
Conjugate, 10x concentrate	1 x 1.5 ml
TMB Substrate, ready to use	1 x 12 ml
Stop Solution, ready to use	1 x 12 ml
Reconstitution Buffer, ready to use	1 x 1.5 ml
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Intended use

cattletype IFN-gamma is an ELISA for the identification of ruminant interferon gamma (IFN-γ). Samples of plasma or cell culture supernatant can be used after stimulation with disease-specific antigens.

For laboratory use only. Not for use in diagnostic procedures.

Symbols

	Legal manufacturer
	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
	Catalog number
	Material number
	For ruminant samples

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of cattletype IFN-gamma is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the cattletype IFN-gamma should be stored at 2-8°C and are stable until the expiration date stated on the label. Wash Buffer (20x), Sample Diluent and Stop Solution may be stored at 2-25°C.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request under **compliance@indical.com**.

CAUTION: The Stop Solution contains 0.5 M maleic acid.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Introduction

Interferon gamma (IFN-γ) is produced predominantly by TH1-cells, as part of the adaptive immune response after contact with a specific antigen.

The cattletype IFN-gamma test kit has been designed for the identification of the inflammation marker IFN-γ from ruminants in plasma or cell culture supernatant after stimulation with specific antigens. This test includes two different monoclonal antibodies against ruminant IFN-γ, as well as native IFN-γ, serving as positive control. The results will be calculated based on this standardized positive control. This guarantees reproducibility in different test runs and batches.

Principle

The cattletype IFN-gamma is a sandwich ELISA. The microtiter plate is coated with an anti-ruminant IFN-γ monoclonal antibody (mab). During sample incubation, IFN-γ that is present in the sample binds to the immobilized antibodies. Unbound material is removed by rinsing. The anti-ruminant IFN-γ mab HRP conjugate binds to the antigen-antibody-complex. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 15 minutes. In the presence of IFN-γ within the sample, HRP catalyzes a blue color development, which turns yellow after adding the Stop Solution. The optical density (OD) is measured in a spectrophotometer. The OD values correlate with the concentration of IFN-γ in the sample.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Beakers
- Measuring cylinders
- Incubator
- Pipets (adjustable)
- Multichannel pipets (adjustable)
- Aluminum or adhesive foil for covering the Test Plate
- Optional: Device for delivery and aspiration of Wash Buffer
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

Important notes

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight when performing the test.
- Components of the test kit should not be contaminated or mixed with components from other batches.
- Do not use the components of the test kit past the expiration date.
- Water from ion-exchange systems used for diluting the Wash Buffer (10x) may interfere with the assay if not pure enough. Use double-distilled water or highly purified water (Milli-Q®).
- For accurate test results, it is essential to use clean glassware and to pipet and rinse carefully and strictly adhere to the incubation times when performing the test.

Protocol: ELISA test procedure

Important points before starting

- Please read „Important notes“ on page 8 before starting.

Things to do before starting

- Bring reagents to room temperature (18-25°C) immediately before use. In case of precipitated salt crystals in the Wash Buffer (20x), dissolve by gentle swirling and warming.
- Dilute Wash Buffer (20x) 1:20 in distilled water. For example, for one Test Plate dilute 25 ml Wash Buffer (20x) in 475 ml distilled water and mix.
- Set the incubator to 37°C.
- Dilute Conjugate (10x) 1:10 in Sample Diluent. For example, for one test plate dilute 1 ml Conjugate in 9 ml Sample Diluent and mix.
- Reconstitute the freeze-dried Positive Control using the Reconstitution Buffer provided with the kit. The volume, which needs to be added, is mentioned on the label of each vial. Wait approximately 5 minutes and mix gently but thoroughly. Ensure complete resolubilization.

  Once re-suspended, the Posiitive Control can be stored:

  - For 1 week at 2-8°C.
  - For longer term storage, aliquot and freeze (< -18°C).

    Each aliquot can undergo three freeze-thaw-cycles without loss of activity and can be stored for 6 months.
- **Optional**: If required, (activated and non-activated) samples can be diluted prior to analysis. Dilute Negative Control (in duplicates), Positive Control (in duplicates) and samples to be tested 1:2 in

Sample Diluent (e.g., dilute 35µl sample in 35µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Use a fresh pipet tip for each sample.

Procedure

1. If using samples that were diluted prior to analysis, go to step 1a. If samples should be diluted in the Test Plate, go to step 1b.

1a. Pipet 50 µl each of the prediluted Negative Control (in duplicates), prediluted Positive Control (in duplicates) and prediluted samples into the wells of the Test Plate. Proceed to step 2.

Note: Record the positions of the controls and samples in a test protocol. We recommend use of a multichannel pipet for sample transfer. Cover the Test Plate with aluminum or adhesive foil.

1b. Pipet 25 µl Sample Diluent into each well of the Test Plate. Pipet 25 µl each of the undiluted Negative Control (in duplicates), undiluted Positive Control (in duplicates) and undiluted samples into the appropriate wells of the Test Plate.

Note: Record the positions of the controls and samples in a test protocol. Cover the Test Plate with aluminum or adhesive foil.

2. Mix the samples by gently shaking the plate for 2 min (± 1 min) at room temperature (18-25°C). Incubate for 60 min (± 5 min) at 37°C (± 2°C) in an incubator.
3. Remove solution from the wells by aspiration or tapping.
4. Rinse each well 6x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
5. Pipet 100 µl of prepared Conjugate to each well and incubate for 60 min (± 5 min) at 37°C (± 2°C) in an incubator. Cover the Test Plate with aluminum or adhesive foil.
6. Remove solution from wells by aspiration or tapping.

7. Rinse each well 6x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
8. Pipet 100 µl TMB Substrate Solution to each well.
9. Incubate for 15 min (± 2 min) at room temperature (18-25°C) in the dark. Begin timing after the first well is filled.
10. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.
11. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.

    Measuring at a reference wavelength (620–650 nm) is optional.

Data interpretation

Validation criteria

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be > 0.5.
- The ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) must be greater than 3 ($OD_{PC}/OD_{NC} > 3$).

In case of invalid assays, the test should be repeated after carefully reading the instructions for use.

Calculation

Calculate the MV of the measured OD for the Positive Control (PC).

The S/P ratio of interferon in the sample (S/P%) is calculated according to the following equation:

$$S/P\% = \frac{OD_{activated} - OD_{non\text{-}activated}}{OD_{PC} - OD_{NC}} \times 100$$

Interpretation of the results

- Samples with an S/P% < 35% are negative.
  No specific IFN-γ production, induced by the tested antigen, could be identified.
- Samples with an S/P% ≥ 35% are positive.
  Specific IFN-γ production, induced by the tested antigen, could be identified.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.

Visit **www.indical.com** for more information about bactotype, cador, cattletype, flocktype, pigtype and virotype products.

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Change index

Handbook	Version	Change
HB-1891-EN-004	April 2019	correction of typing error, page 3
HB-1891-EN-003	November 2018	INDICAL design

Quick guide for cattletype IFN-gamma

Sample dilution:

Plasma, cell culture supernatant 1:2, mix well

Step	Protocol
1. Sample	25 µl Sample Diluent + 25 µl sample
2. Mix	2 min RT, gentle shaking
3. Incubation	60 min at 37°C
4. Wash	6 x 300 µl
5. Conjugate	100 µl/ well
6. Incubation	60 min at 37°C
7. Wash	6 x 300 µl
8. TMB	100 µl/ well
9. Incubation	15 min at RT
10. Stop	100 µl/ well
11. Read	450 nm

Data interpretation

Result	Induced IFN-γ production	Status
S/P% < 35%	No	Negative
S/P% ≥ 35%	Yes	Positive