Bovine Interferon-γ Specific ELISA Assay Kit

MCA5638KZZ

FOR INVITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES

Instructions for Use

Intended Use

This ELISA kit is a sandwich enzyme immunoassay designed for the quantitative determination of bovine interferon gamma in bovine serum, plasma and culture supernatant.

Summary & Explanation

IFN-γ (Interferon-gamma) is produced by activated T cells and NK cells. It is a proinflammatory cytokine that activates macrophages and endothelial cells, but it also regulates immune responses by affecting APC and T and B cells. Production of IFN-γ (Interferon-gamma) by helper T cells as well as cytotoxic T cells is a hallmark of the TH1-type phenotype, thus high-level production of IFN-γ (Interferon-gamma) is typically associated with effective host defence against intracellular pathogens.

Principle of the Method

This assay is based on a sandwich ELISA using two different mouse anti-bovine IFN-γ monoclonal antibodies and recombinant bovine IFN-γ as a standard.

Performance

Assay range - 0.025-50ng/ml in bovine serum, plasma and culture supernatant.
Sensitivity - The Limit of Detection of the IFN-γ is typically < 0.025 ng/ml

Contents of the Bovine IFN-γ Kit

Each kit (480 tests) contains the following reagents and consumables:

Item	Quantity
96 well microtiter plate	5
Microplate adhesive sealer	5
Coating Buffer 5x concentrated	1
Coating Antibody (clear top)	1
Bovine IFN-γ standard 400ng/ml, lyophilised	1
Detection Antibody (blue top)	1
Streptavidin:HRP Conjugate (brown top)	1
HRP Substrate (ready to use)	1

Reagents and Equipment required but not supplied

Blocking solution - available from Bio-Rad Cat No BUF032A/B/C, or see Appendix 1 for buffer recipe
Wash buffer - available from Bio-Rad Cat No BUF031A/B/C, or see Appendix 1 for buffer recipe
Stop solution - see Appendix 1 for recipe
Distilled or deionised water
Plate reader with 450nm reading capability
Pipettes for dispensing up to 250µl
Tubes for preparing standard and sample dilutions

Preparation of Reagents

This ELISA kit is a sandwich enzyme immunoassay designed for the quantitative determination of bovine interferon gamma in bovine serum, plasma and culture supernatant.

NOTE: Bring all reagents to room temperature before use.

Item	Preparation
Coating Buffer (MCA5638KZZCoat)	Dilute the stock solution 1:5 in distilled water. For each 96 well plate add 2ml coating buffer to 8ml distilled water. If crystals are present in the stock solution warm gently to 37ºC to dissolve prior to diluting.
Coating Antibody (MCA5638KZZA)	Prepare a 1:200 dilution of this antibody in diluted Coating Buffer. For each 96 well plate 50µl coating antibody diluted in 10mls buffer is sufficient.
IFN-γ Standard (MCA5638KZZB)	Add 625µl distilled water to the standard and mix gently but thoroughly for 5 minutes. Once resuspended, the standards can be stored at 2-8ºC for two months. For longer term storage, freeze at -20ºC; before use thaw the vial, bring to room temperature, and mix well.
Detection Antibody (MCA5638KZZC)	Prepare a 1:500 dilution of this antibody in Wash Buffer. For example for a 96 well plate 20µl of detection antibody in 10ml of buffer is sufficient.
Streptavidin:HRP Conjugate (MCA5638KZZD)	Dilute 1:1000 of Streptavidin:HRP Conjugate in wash buffer, for example 50µl in 50ml wash buffer, which is sufficient for 5 plates.
Wash Buffer	Prepare the required amount of wash buffer assuming each well requires 4.5mls wash buffer for this assay. Unused wash buffer can be stored at 4ºC for up to one month.
HRP Substrate (MCA5638KZZTMB)	This is a ready to use solution. To avoid contamination avoid contact with metals or exposure to light. Pipette the required amount of solution into a clean tube and do not return any residual solution back to the original bottle

Sample collection and storage

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8°C within 30 minutes of collection. Store samples at -20°C or -80° C.
Avoid repeated freeze-thaw cycles.

Cell culture supernatants and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note: Serum, plasma, and cell culture supernatant samples to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20° C (≤ 3 months) or -80° C (≤ 6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.
It is recommended that all samples be assayed in duplicate.
DO NOT USE HEAT-TREATED SPECIMENS.

Assay Protocol

Step 1	Calculate the number of wells required (samples must be tested at least in duplicate) and include 18 wells for a standard curve.
Step 2	Carefully pipette 100µl of diluted coating antibody into the wells.
Step 3	Cover the plate with an adhesive plate sealer and incubate at room temperature for one hour or 2-8°C overnight.
Step 4	Prepare a series of tubes labelled 1-9 and make serial dilutions of the reconstituted standard following the dilution table below. Mix thoroughly between steps.

Tube #	Standard	Add
1	50ng/ml	50µl Reconstituted standard + 350µl wash buffer
2	12.5ng/ml	150µl from tube 1 + 450µl wash buffer
3	6.25ng/ml	250µl from tube 2 + 250µl wash buffer
4	3.13ng/ml	250µl from tube 3 + 250µl wash buffer
5	1.56ng/ml	250µl from tube 4 + 250µl wash buffer
6	0.78ng/ml	250µl from tube 5 + 250µl wash buffer
7	0.2ng/ml	150µl from tube 6 + 450µl wash buffer
8	0.1ng/ml	250µl from tube 7 + 250µl wash buffer
9	0.025ng/ml	100µl from tube 8 + 300µl wash buffer

Assay Protocol continued

Step 5	Remove the plate sealer and wash the wells three times with wash buffer. Aspirate the last wash and gently tap the plate upside down on a paper towel. Do not allow the wells to dry.
Step 6	Add 200µl blocking buffer per well, seal the plate with a plate sealer and incubate for 1 hour at room temperature.
Step 7	Remove the plate sealer and wash the wells three times with wash buffer. Aspirate the last wash and gently tap the plate upside down on a paper towel. Do not allow the wells to dry.
Step 8	Add 100µl of diluted standards to the wells designated for the standard curve, and add sample (diluted in wash buffer) to the other wells, all in duplicate.
Step 9	Seal the plate with a plate sealer and incubate at room temperature for 1 hour.
Step 10	Remove the plate sealer and wash the wells three times with wash buffer. Aspirate the last wash and gently tap the plate upside down on a paper towel. Do not allow the wells to dry.
Step 11	Add 100µl of pre-diluted detection antibody to each well of the plate. Seal the plate with a plate sealer and incubate at room temperature for 1 hour.
Step 12	Remove the plate sealer and wash the wells three times with wash buffer. Aspirate the last wash and gently tap the plate upside down on a paper towel. Do not allow the wells to dry.
Step 13	Add 100µl of pre-diluted Streptavidin:HRP Conjugate to each well of the plate. Seal the plate with a plate sealer and incubate at room temperature for 1 hour.
Step 14	Remove the plate sealer and wash the wells three times with wash buffer. Aspirate the last wash and gently tap the plate upside down on a paper towel. Do not allow the wells to dry.
Step 15	Add 100µl of TMB substrate to each well and agitate the plate.
Step 16	Incubate the plate for 15 minutes before adding 100µl Stop solution to each well.
Step 17	Read the plate at 450nm.
Result	Fig 1 shows a typical calibration curve.

Precaution
The stop solution suggested for use with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.

Appendix 1: Buffer Recipes

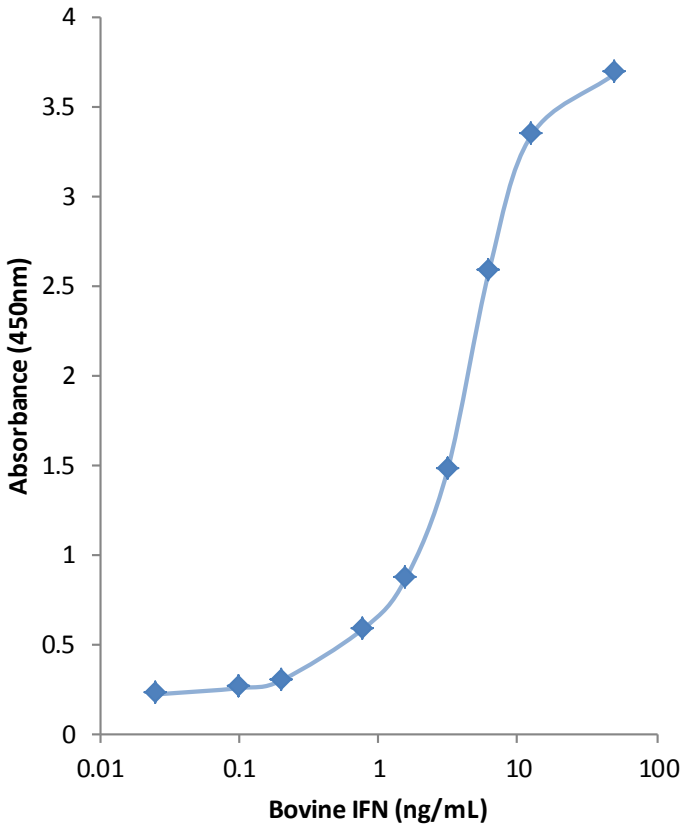
Wash Buffer: 0.2M NaCl, 0.05% Tween-80
Blocking Buffer: 4% BSA in PBS
Stop Solution: 0.2M Sulphuric acid

For product support or advice please contact:

Technical inquiries can be made by telephone, fax, or email – simply choose the most convenient option. Please provide as many details as possible, so that we can deal with your request quickly and efficiently.

Region	Phone	Fax	Email
North America	+1 800 265 7376	+1 919 878 3751	antibody_tech_us@bio-rad.com
United Kingdom	+44 1865 852 733	+44 1865 852 739	antibody_tech_uk@bio-rad.com
Other Countries	+49-211 23 95 64 50	+49 211 23 95 64 99	antibody_tech_uk@bio-rad.com

Fig 1. Typical Calibration Curve produced by the Bovine Interferon-γ Specific ELISA Assay Kit.



Bio-Rad Bovine Interferon Gamma Quick Guide