III PRINCIPLE OF THE BOVINE IFN-γ EASIA TEST

The Bovine IFN-γ EASIA is a 2 step Enzyme Immunometric Assay performed in microplates and is designed to measure bovine IFN-γ in biological samples.
In the first step, IFN-γ from either controls or samples reacts with the capture monoclonal antibody fixed to the well. Following a washing step, horseradish peroxidase anti-bovine IFN-γ monoclonal antibody conjugate is added.
The antibody conjugate binds to the immobilised IFN-γ. The unbound conjugate is washed out and the chromogen solution is added.
The colour development is proportional to the amount of bovine IFN-γ present in samples or controls. Finally, the reaction is stopped and the plate is read spectrophotometrically at 450nm.

IV REAGENTS PROVIDED

Reagents	96 Tests Kit	192 Tests Kit	Reconstitution
1 plate including 96 anti-IFN-γ bovine-coated wells	1 x 96 wells	2 x 96 wells	**Ready** to use
Positive Control in bovine serum containing natural bovine IFN-γ Contains ProClin®300 0.04% (v/v)	1 vial lyophil.	2 vials lyophil.	**Add** 0.5 ml distilled water
Negative Control in bovine serum containing natural bovine IFN-γ. Contains ProClin®300 0.04% (v/v)	1 vial lyophil.	2 vials lyophil.	**Add** 0.5 ml distilled water
Concentrated Anti-bovine IFN-γ HRP conjugate in buffered solution. Contains Micr-O-Protect™ 0.25% (v/v)	1 vial 0.75ml	2 vials 0.75 ml	**Dilute** with conjugate buffer according to Table 2
Conjugate buffer. Contains Micr-O-Protect™ 0.5% (v/v)	1 vial 12 ml	2 vials 12 ml	**Ready** to use
Incubation buffer. Contains Micr-O-Protect™ 0.5% (v/v)	1 vial 6 ml	2 vials 6 ml	**Ready** to use
Chromogen : TMB (Tetramethylbenzidine)	1 vial 25 ml	1 vial 25 ml	**Ready** to use
Stop Solution	1 vial 25 ml	2 vials 25 ml	**Ready** to use
Super wash (25 x concentrate) with preservatives	1 vial 30 ml	2 vials 30 ml	**Dilute** 1 vol with 25 volume of distilled water or the whole solution into 750 ml of distilled water

V PRECAUTIONS AND WARNINGS

1. The bovine blood component included in this kits have been screened for mycoplasma and viruses and found negative.
   The bovine components used in this kit originate from animals coming from countries where Bovine Spongioform Encephalitis has not been reported.
2. Avoid skin contact with Stop Solution and Chromogen solution. In case of contact, wash thoroughly with water.
3. Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
4. Do not pipette liquids by mouth.
5. Do not use kit components beyond the expiration date.
6. Bring all the reagents and specimens to room temperature (18-24°C).
7. Use a clean disposable plastic tip for each reagent, control or specimen addition in order to avoid cross-contamination.
8. Avoid pipettes with metal parts for the dispensing of the chromogen, substrate solution and stop solution.
9. During the incubation and colour development step, avoid direct sunlight exposure on the microplate.

VI EQUIPMENT AND SUPPLIES REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water high grade quality
2. Precision pipette : 0.05, 0.1, 0.25 and 1 ml and multiple delivery pipetting device
3. Vortex mixer and magnetic stirrer.
4. Horizontal microtiter shaker capable of 700 rpm ± 100 rpm, microtiter plate washing device.
5. Microplate reader fitted with 450nm and any other blank filter from 630nm up to 750 nm.

VII REAGENT PREPARATION

1. Negative and positive controls

Reconstitute the lyophilized Controls to the volume specified on the vial label (0.5 ml) with distilled water. Allow them to remain undisturbed until completely dissolved, then mix by gentle I inversion and finally on a vortex mixer.

2. HRP Conjugate Working Solution

Depending on the number of wells to be used, dilute the concentrated conjugate with the conjugate buffer in a clean glass vial according to Table 2.

Table 2 : Working Conjugate préparation

Number of wells (n)	Concentrated HRP Conjugate (ml)	Conjugate Buffer (ml)	Working Volume (ml)
16	0.09	1.71	1.8
32	0.18	3.42	3.6
48	0.27	5.13	5.4
96	0.54	10.26	10.8
192	1.08	20.52	21.6

To prepare any other working volume of conjugate, mix one volume of Concentrated HRP Conjugate with 19 volumes of Conjugate Buffer.

3. Working Washing Solution

Dilute 6 ml of Super-Wash (25x concentrate) with 150 ml of distilled water or all the content of the vial with 750 ml of distilled water.

VIII STORAGE AND SHELF LIFE OF REAGENTS

A. UNOPENED reagents

. Store the unopened vial at 2-8°C till expiration date printed on the kit.

B. USED reagents

1. The Concentrated HRP Conjugate must be stored at 2-8°C till expiration date.
2. The reconstituted Controls are stable for 4 days at 2-8°C; at -25°C for two months or at -70°C for 1 year.
3. Store the unused strips at 2-8°C in the sealed bag with desiccant.
4. The Working Conjugate Solution is stable for 3 days at 2-8°C.
5. The Working Washing Solution is stable 3 days at room temperature.

IX SPECIMEN COLLECTION, PREPARATION AND STORAGE

A. Specimen Collection

The Bovine IFN-γ EASIA may be used to measure bovine IFN-γ in serum, plasma, cell culture medium or plasma from stimulated whole blood (see reference 2, 3 and 5).
Avoid grossly lipemic or hemolysed samples.

B. Storage

Until use, plasma samples should be kept at -25° C for up to 2 months. For longer periods of time, store samples at -70°C. Samples with low protein levels such as culture medium should be stored at - 70°C.

C. Sample Dilution

Plasma samples containing high levels of IFN-γ should be diluted with negative control or with bovine plasma without any residual IFN-γ level. Culture medium containing high levels of IFN-γ should be diluted with culture medium supplemented with 10% FCS.

X BOVINE IFN-γ EASIA PROCEDURE

A duplicate assay on samples is recommended.

1. Select the required number of strips for the run.
2. Secure the strips in the holding frame.
3. Pipette 100 µl of Controls and Samples into the appropriate wells.
4. Pipette 50 µl of Incubation Buffer into each well.
5. Incubate the plate for 1h at room temperature on a horizontal shaker set at 700 ± 100 rpm.
6. Aspirate the contents of each well.
7. Wash the plate 3 times with 400 µl of Working Washing Solution.
8. Eliminate the remaining liquid drops by tapping the plate on an absorbent paper.
9. Pipette 100 µl of Working Conjugate Solution into each well.
10. Incubate the plate for 1h at room temperature on a horizontal shaker set at 700 ± 100 rpm.
11. Aspirate the contents of each well.
12. Wash the plate 3 times with 400 µl of Working Washing Solution.
13. Eliminate the remaining liquid drops by tapping the plate on an absorbent paper.
14. Dispense 100 µl of the Chromogen (TMB) solution into each well.
15. Incubate the plate for 15 min at room temperature on a horizontal shaker set at 700 ± 100 rpm.
16. Dispense 200 µl of Stop Solution into each well to stop the colour development.
17. Read the plate at 450 nm against any reference filter from 630 to 750 nm. Record the absorbance values.

XI ASSAY CUTOFF CALCULATION

Average the OD of the Negative Control. The Assay Cut-off is calculated as: mean OD_{NC} + 0.15.
Example :

Negative Control :		0.023 0.033
Mean :		0.028
Assay Cut-off :	0.028 + 0.15	0.178

XII RESULTS INTERPRETATION

. Samples yielding mean OD below the Assay Cut-off are NEGATIVE for IFN-γ.
. Samples yielding mean OD above the Assay Cut-off are POSITIVE for IFN-γ

XIII QUALITY CONTROL

The average absorbance value of the Negative Control must be less than 0.075 OD.
The Positive Control average OD must be above the Assay Cutoff and possess a ratio of $\frac{OD}{CutOff}$ greater than 5.

XIV PERFORMANCE CHARACTERISTICS

A. Specificity

Individual plasma samples from 1882 apparently healthy cows after whole blood stimulation by bacterial preparation were analysed by the EASIA IFN-γ method.
Of these samples, 1844 were below cut-off for a specificity of 98%.

B. Sensitivity

No source of purified recombinant or natural bovine IFN-γ is currently available. Sensitivity is therefore estimated based on the ability to obtain specific signals from samples stimulated under conditions known to give rise to IFN-γ.
Stimulation of 46 “clinical ill” bovine whole blood samples by bacterial preparation yielded signals above Assay Cut-off in 96% of the samples.

C. Precision

Within - Assay			Between - Assay		
n	OD mean ± SD	CV %	n	OD mean ± SD	CV %
16	1.31 ± 0.04	3	16	1.33	2.6

D. Dilution

Positive Control	mOD
Undiluted	1660
1/2	858
1/4	435
1/8	228
1/16	137
1/32	74
1/64	46

Dilutions were performed with negative control as diluent Linear regression analysis of sample O.D. versus the expected O.D. yielded a corrélation coefficient of 0.999. These data show that a semi-quantitative assay can be constructed if desired.

XV LITERATURE REFERENCES

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XVI SUMMARY OF ASSAY PROCEDURE

	Controls (µl)	Plasma (µl)	Diluted culture medium (µl)
Positive control	100	-	-
Negative control	100	-	-
Sample	-	100	-
Diluted culture médium	-	-	100
Incubation buffer	50	50	50
Incubate for 1 hour at RT on horizontal shaker set at 700 ± 100 rpm **Aspirate** the content of each well **Wash** 3 times with 400 µl of working washing buffer			
Working HRP conjugate	100	100	100
Incubate for 1h at RT on horizontal shaker set at 700 ± 100 rpm **Aspirate** the content of each well **Wash** 3 times with 400 µl of working washing buffer			
Chromogenic solution (TMB)	100	100	100
Incubate for 15 min at RT on horizontal shaker set at 700 ± 100 rpm			
Stop Solution	200	200	200
Read the plate on a microplate reader set at 450 nm versus 630 nm to 750 nm reference filter. **Record** the absorbance and calculate cutoff.			

BioSource Catalogue Number : KBC1231 / KBC1232	P.I. Number : 1700579	Date of issue : 09 February 2000

Before use, read this Package Insert.

Bovine I F N-γ E A S I A

For research use only. Not for use in diagnostic procedures.
An enymatic immunoassay for the qualitative measurement of bovine interferon gamma (IFN-γ)
in plasma, cell culture medium or other biological fluids.

I GENERAL INFORMATION

A. Proprietary Name : BioSource Bovine IFN-γ EASIA

B. Catalogue Number : KBC1231 : 96 determinations
KBC1232 : 2 x 96 determinations

C. Manufactured by : BioSource Europe S.A.
Rue de l'Industrie,8 B-1400 Nivelles
Belgium.

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II INTRODUCTION

Interferon-Gamma, (IFN-γ, type 2, Immune IFN) is structurally and functionally distinct from type 1 (alpha/beta) interferons and binds to a separate receptor. Only one IFN-γ gene has been identified,coding for a 146 AA protein that is post-transitionally processed into two glycosylated species of 20 and 25 from Kd. Native IFN-γ is pH2-labile, highly basic, and can aggregate to form dimers that are biologically active.
IFN-γ is a lymphokine produced by activated T (and NK) cells. Despite its clear antiviral and cellular growth-regulating activities, its immunomodulatory properties are believed to be the most important. IFN-γis the principal activator of macrophage function (Macrophage activating factor, MAF), and it also regulates the pathway of differentiation of myeloïd cells. IFN-γplays an important role in the growth and differentiation of cytotoxic (and possibly suppressor). T cells, activates NK cells and acts as a B cell maturation factor.
IFN-γ regulates Ig isotype production and inhibits IgE responses. One of the modes of action of IFN-γ is to induce the expression of membrane proteins, such as class 1 and class 2 MHC antigens and adhesion molecules on various cell types, high affinity Fc receptors for IgG on myelomonocytic cells, etc. Integrated in the cytokine network, IFN-γ interacts with other cytokines, in either a synergistic (e.g.TNF) or antagonistic (e.g. IL-4) way. The precise role of IFN-γin diseases and therapy is still poorly defined. Clearly, it is involved in the defence against parasites, intracellular pathogens and possibly tumour cells. The BioSource bovine IFN-γ EASIA provides a sensitive tool for the qualitative measuring in vitro IFN-γ in bovine species.