Mycobacterium bovis Gamma Interferon Test Kit for Cattle
BOVIGAM® 2G

An *in vitro* diagnostic test kit for detection of bovine tuberculosis infection in cattle

63330: 960 Test Wells – Ten (10) Microplate Test Kit

Version 4.3_e

Package Insert

For *in vitro* veterinary diagnostic laboratory use only.
Store at 5±3°C
Product No: 63330

Introduction

BOVIGAM® 2G is a rapid *in vitro* blood-based assay of cell mediated response to *M. bovis* PPD tuberculin for the diagnosis of bovine tuberculosis infection in cattle.

Tuberculin PPD antigens or synthetic antigen cocktails are presented to lymphocytes in whole blood culture. The production of IFN-γ from cells is then detected using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). Lymphocytes from cattle not infected with *M. bovis* do not produce IFN-γ. Therefore, detection of IFN-γ correlates to *M. bovis* infection.

Kit Components

Product Number
Ten (10) Microplate Test Kit: 63330

Component 1
Microplate coated with antibody to IFN-γ
63330: 10 x 96 well plates with lids
Ready-to-use.

Component 2
Positive Bovine IFN-γ Control
63330: 2 x 1 ml
Freeze-dried. Reconstitute with deionised or distilled water.

Component 3
Negative Bovine IFN-γ Control
63330: 2 x 1 ml
Freeze-dried. Reconstitute with deionised or distilled water.

Component 4
Green Diluent: Sample Diluent Buffer
63330: 1 x 60 ml
Ready-to-use.

Component 5
Wash Buffer – 20X Concentrate
63330: 2 x 200 ml
Dilute with deionised or distilled water.

Component 6
Conjugate – 250X Concentrate (Horseradish peroxidase labelled anti-bovine IFN-γ)
63330: 1 x 0.6 ml
Freeze-dried. Reconstitute with deionised or distilled water

Component 7
Blue Diluent: Conjugate Diluent Buffer
63330: 1 x 125 ml
Ready-to-use.

Component 8
Enzyme Substrate Solution
Contains H_2O_2.
63330: 1 x 125 ml
Ready-to-use.

Component 9
Enzyme Stopping Solution
63330: 1 x 75 ml
(0.5M H_2SO_4)
Ready-to-use.

Store kit at 5±3°C. Bring all reagents except Conjugate Concentrate to room temperature (22±3°C) before use. Return to 5±3°C immediately after use.

Material required but not provided

BLOOD COLLECTION
1. Lithium heparin Vacutainers: 1 / animal
2. 18G Vacutainer needles — 1 inch: 1 / animal
3. Needle holders: 2-3 / blood collector

BLOOD CULTURE
1. Sterile 96-well tissue culture trays (flat bottom): 1 / 15 animals, e.g. TPP; #92096;TC or comparable (http://www.tpp.ch/page/produkte/09_zellkultur_testplatte.php)
2. Sterile phosphate buffered saline: 100 µl / animal (0.01M, pH 7.2)
3. Antigens for stimulation
4. RPMI 1640 Medium, e.g. Gibco 72400-013 or comparable (http://www.invitrogen.com/site/us/en/home/support/Product-Technical-Resources/media_formulation.123.html)

PLASMA HARVESTING
1. Tips to fit 25-1000 µl pipette: 3 / animal
2. 1 ml microtubes in 96-well format racks and caps for plasma storage: 1 rack / 15-30 animals

BOVINE IFN-γ EIA
1. Tips to fit 8 / 12 channel pipette: 3 / animal
2. Various polypropylene tubes, EIA reagent troughs, and tips

Equipment required but not provided

1. 37°C humidified incubator
2. Accurate, replaceable-tip variable-volume pipettes (to deliver up to 1 ml)
3. Graduated 1, 5 and 10 ml pipettes
4. Measuring cylinders — 100 ml, 1 l and 2 l
5. Deionised or distilled water — 6 l
6. 8 or 12 channel pipette (to deliver 50 µl, 100 µl, and 300 µl)
7. Microplate shaker
8. Microplate/strip washer
9. Microplate reader. This reader MUST be fitted with a 450 nm and 620-650 nm filters
10. Centrifuge for microplates

Antigens and stimulation control deliverable but not provided

1. **Peptide Cocktail Prionics® PC-EC**
   7600100: 1 x 3 ml (lyophilized), 120 samples

   For high specific testing by acceptance of a lower sensitivity than Tuberculin PPD use.

2. **Peptide Cocktail Prionics® PC-HP**
   7600105: 1 x 3 ml (lyophilized), 120 samples

   Combines the advantages of Tuberculin PPD testing (highest sensitivity) and Peptide Cocktail Prionics® PC-EC testing (highest specificity) resulting in a high sensitivity *as well as* high specificity. Instructions for use of both Peptide Cocktails: see enclosed detailed Package Insert of the respective product.

3. **Pokeweed Mitogen PWM**
   5108777: 1 x 3.2 mg (lyophilized), up to 2320 stimulations possible
   To be reconstituted with 3.2 ml RPMI 1640 Medium.

4. **Bovine Tuberculin PPD 3000**
   7600060: 1 x 5 ml
   Avian Tuberculin PPD 2500
   7600065: 1 x 5 ml

Preparation of Reagents

1. **ANTIGENS**
   Bovine Tuberculin PPD 3000 and **Avian Tuberculin PPD 2500** (stimulation antigens) obtained through Prionics AG.
   Mix thoroughly before use. Dilute the stimulation antigens as follows (see also enclosed detailed Package Insert of the product):

   For stimulation in 96-well cell culture plates:

   Dilute 110 µl **of Bovine Tuberculin PPD 3000 (30'000 IU/ml)** in 890 µl of RPMI medium for final assay concentration of 300 IU/ml.

   Dilute 110 µl of **Avian Tuberculin PPD 2500 (25'000 IU/ml)** in 890 µl of RPMI medium for final assay concentration of 250 IU/ml.

   Preparation schedule for other PPD might be different.

   Prionics recommends implementing also a Pokeweed Mitogen control to demonstrate viability of the cells:
   The **Pokeweed Mitogen** is lyophilized and has to be stored at 5±3°C. Reconstitute with 3.2 ml RPMI1640 Medium to a stock solution of 1 mg/ml. This 3.2 ml stock solution should be frozen in suitable aliquots (-20 °C to -25 °C).

   For stimulation purposes a suitable aliquot of the stock solution (1 mg/ml) is thawed. In order to get a final assay concentration of 5 µg/ml, dilute 55 µl stock solution (1 mg/ml) in 945 µl RPMI 1640 Medium.

2. **PLATES**
   Allow plate(s) to equilibrate to room temperature (for at least 30 minutes) before unsealing plastic pouch.

3. **POSITIVE AND NEGATIVE CONTROLS**
   Reconstitute appropriate vials with 1 ml of deionised or distilled water.

   Ensure complete resolubilisation and aliquot appropriately for SINGLE USE only. Reconstituted controls are stable for 4 weeks at 4°C, however, they may be stored at **-70°C** for several months, but MUST be brought to room temperature and mixed thoroughly before use.

4. **GREEN DILUENT**
   Bring to room temperature and mix thoroughly. Use undiluted as Sample Diluent Buffer.

5. **CONJUGATE**
   The **Conjugate - 250X Concentrate** is provided in freeze-dried form **and has to be reconstituted with 0.6 ml** of deionised or distilled water.

Mixing should be performed with a minimum of frothing.
Conjugate -250X Concentrate MUST be kept at 5±3°C at all times and used WITHIN 3 months of reconstitution.
NOTE: Excessive frothing of conjugate may cause denaturation and reduce its performance in the EIA.

6. **BLUE DILUENT AND CONJUGATE DILUTION**
The Blue Diluent is supplied pre-diluted and ready to use. Prepare working solution Conjugate Reagent combining appropriate volumes of working solution Blue Diluent and Conjugate - 250X Concentrate as set out in the Reagent Preparation Table (Table 1). Mix thoroughly but gently. Avoid frothing. Return any unused Conjugate - 250X Concentrate to 5±3°C immediately after use.

7. **WASH BUFFER**
Prepare working solution Wash Buffer by adding one part 20X Concentrate to 19 parts deionised or distilled water. Mix thoroughly. Working solution Wash Buffer may be stored at room temperature for up to 2 weeks. Unused Wash Buffer 20X Concentrate should be returned to 5±3°C after use.

NOTE: Wash Buffer - 20X Concentrate may contain salt crystals. Re-dissolve crystals by warming to 37°C. Mix thoroughly before dilution.

8. **ENZYME SUBSTRATE SOLUTION**
Bring the Enzyme Substrate Solution to room temperature. The solution should be colourless. Discard if blue colouration occurs.

NOTE: If possible use plastic polypropylene disposable containers sterilised by irradiation to prepare the enzyme substrate solution.

DO NOT USE POLYSTYRENE CONTAINERS OR PIPETTES. Any glassware used with the Enzyme Substrate Reagents should be rinsed thoroughly with 1N H_2SO_4 or HCl followed by at least three washes of deionised or distilled water, ensuring no acid residue remains on the glassware.

9. **SAFE DISPOSAL OF REAGENTS**
All waste and unused portions of prepared reagents should be disposed of in accordance with State and local requirements.

REAGENT PREPARATION TABLE FOR DILUTING CONJUGATE - TABLE 1

Number of Plates	Volume of Conjugate - Concentrate (250X)	Volume of working strength Blue Diluent
1	0.048 ml	12 ml
2	0.096 ml	24 ml
3	0.140 ml	35 ml
4	0.180 ml	45 ml
5	0.220 ml	55 ml
6	0.260 ml	65 ml
7	0.300 ml	75 ml
8	0.340 ml	85 ml
9	0.380 ml	95 ml
10	0.420 ml	105 ml

Procedural Notes

1. All test plasmas and reagents except the Conjugate - 250X Concentrate MUST be brought to room temperature (22±3°C) before use. Thawed test samples should be mixed thoroughly by carefully vortexing each tube. DO NOT WARM ABOVE 37°C.

2. All kit components are to be stored at 5±3°C. Return to 5±3°C immediately after use. Working solution Wash Buffer may be stored at room temperature (22±3°C) for up to 2 weeks.

3. The Conjugate - 250X Concentrate must be left at 5±3°C at all times.

4. Complete reconstitution of freeze dried components (Positive and Negative Bovine IFN-γ Control and Conjugate - 250X Concentrate) is essential for valid tute reagents and allow vials to sit for at least 15 minutes, then mix by gently inverting each vial 4 or 5 times. A roller-rocker apparatus may be used. Mix again just prior to use.

NOTE: It is important that high quality deionised or distilled water is used to reconstitute and dilute reagents. Reagents such as horseradish peroxidase are readily inactivate by pollutants common in laboratory water supplies.

5. Once the assay has been started it should be completed without interruption.

6. Use a separate disposable tip for each sample to prevent cross contamination.

7. Test plasmas from individual animals should be added simultaneously to EIA wells using an 8 or 12 channel pipette.

8. EIA plates should be incubated on a plate shaker at a setting of 600 rpm ± 50 rpm to minimise inter-well variations. If EIA plates are not incubated on a plate shaker, plates should not be incubated directly on the bench, but elevated on an inverted test-tube rack (or similar). The cold solid surface may act as a heat sink and lead to the phenomenon commonly known as 'edge effect'.

9. Positive and Negative Bovine IFN-γ Controls should be tested in triplicate in serial wells of columns 4, 5 and 6 (e.g. row C for positive and row D for negative controls).

Test Procedure

STAGE ONE – WHOLE BLOOD CULTURE METHOD

1. **Blood Collection:**
Collect a minimum volume of 2 ml of blood from each animal into a blood collection tube containing heparin as anticoagulant and gently mix blood by inversion several times to dissolve the heparin. Blood samples should be transported to the laboratory at ambient temperature (22±3°C, avoid extremes) and put into culture within 30 hours of collection. Under no circumstances should blood be stored in refrigerator.

2. **Dispensing Blood:**
Blood samples must be evenly mixed before aliquoting. Use a roller-rocker or gently invert tubes about 10 times immediately prior to dispensing.

NOTE: It is important to keep cell damage to an absolute minimum as the test requires viable lymphocytes.

Dispense eight 250 µl aliquots of heparinised blood from each animal into wells of a 96-well tissue culture tray (see Table 2 for recommended layout). This should be performed under aseptic conditions using either sterile disposable pipettes with automatic pipette filler or sterile transfer pipettes.

RECOMMENDED 96-WELL LAYOUT FOR WHOLE BLOOD STIMULATION AND ELISA - TABLE 2

Double Well Testing with Pokeweed Control

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	bP A1	bP A2	bP A3	bP A4	bP A5	bP A6	bP A7	bP A8	bP A9	bP A10	bP A11
B	PC	bP A1	bP A2	bP A3	bP A4	bP A5	bP A6	bP A7	bP A8	bP A9	bP A10	bP A11
C	PC	aP A1	aP A2	aP A3	aP A4	aP A5	aP A6	aP A7	aP A8	aP A9	aP A10	aP A11
D	NC	aP A1	aP A2	aP A3	aP A4	aP A5	aP A6	aP A7	aP A8	aP A9	aP A10	aP A11
E	NC	NL A1	NL A2	NL A3	NL A4	NL A5	NL A6	NL A7	NL A8	NL A9	NL A10	NL A11
F	NC	NL A1	NL A2	NL A3	NL A4	NL A5	NL A6	NL A7	NL A8	NL A9	NL A10	NL A11
G	X	PW A1	PW A2	PW A3	PW A4	PW A5	PW A6	PW A7	PW A8	PW A9	PW A10	PW A11
H	X	PW A1	PW A2	PW A3	PW A4	PW A5	PW A6	PW A7	PW A8	PW A9	PW A10	PW A11

NL=Nil Control Antigen (PBS); aP=Avian PPD; bP=Bovine PPD; PC=positive Control; NC=negative Control; PW=Pokeweed Mitogen; A = Animal

There are additionally 3 different layouts described in this document under "Additional Layouts for Whole Blood Stimulation and ELISA" (see below) also available in Prionics PrioPresto®.

3. **Addition of Stimulation Antigens:**
Add 25 µl of PBS (nil antigen control), diluted working stock of avian PPD and bovine PPD using aseptic technique to the appropriate wells containing the blood previously dispensed in Step 1 of the procedure above. Antigens are best dispensed using a multichannel pipette. The antigens must be mixed thoroughly into the aliquoted blood. Preferably use a microplate shaker to shake each 96-well culture tray at 600 rpm for approximately 1 min. or until the next plate is ready. Avoid frothing of blood.

Add 25 µl of the prepared Pokeweed Mitogen (final assay concentration) to an additional blood containing well per animal and follow the same procedure as described for PBS and PPDs above. The remaining solution has to be discarded and should not be reused. The concentrated stock solution (1 mg/ml) should be thawed only once and not reused.

Optimal performance of this test is dependent on the stimulation antigens being completely mixed in with the blood.

4. **Incubation:**
Incubate tissue culture trays, containing blood and antigens, for 16-24 hours at 37°C in a humidified atmosphere.

5. **Harvesting of Plasma Samples:**
Plasma collection may be facilitated by centrifuging the 96-well trays at 500 g for approximately 10 minutes at room temperature (22±3°C). After the incubation, carefully remove approximately 110 µl from each well of plasma from above the sedimented red cells using a variable-volume pipette (100-300 µl) and transfer pool into separate storage tubes. It is convenient to use 1 ml microtubes in 96-well format storage racks.
Two wells from the same sample of the stimulation plates (for example well A2 and B2) are pooled into plasma storage plate well A2.

Use a convenient plasma storage layout which allows for transfer of plasma into the EIA plate with a 8 or 12 channel pipette. Use a new pipette tip for each plasma sample.

NOTE: It is important to minimise harvest of any cellular material along with plasma. However, contamination of the plasma with a very small amount of erythrocytes during harvesting has no effect on the IFN-γ EIA. Similarly, slight haemolysis of blood samples has little effect on the IFN-γ EIA.

6. **Plasma Storage:**
Plasma may be stored at 5±3°C for up to 7 days if not required for assays on the day of collection. Each microtube must be sealed with an appropriate cap before storage. Label sample racks with all relevant information including date, operator initials, tube contents and animal numbers and herd details. For longer periods, samples may be stored frozen at –20°C for several months.

NOTE: Samples must be allowed to equilibrate to room temperature prior to testing by EIA. Carefully vortex each tube several times immediately prior to assay for IFN-γ.

CAUTION:
Plasma may clot during thawing. Clots do not affect the ELISA, as long as the volume of plasma being aspirated by the pipette is not affected.

STAGE TWO – BOVINE IFN-γ EIA

1. Reconstitute freeze dried components, if required, while equilibrating other reagents according to the guidelines outlined in "Procedural Notes".

3. Add 50 µl of test and control samples to the appropriate wells containing Green Diluent. Control samples should be added last to each plate. Mix thoroughly by vortexing plates for 1 minute on a microplate shaker, or, if not available, by pipetting up and down 5 times.

4. Cover each plate with a lid and incubate at room temperature (22±3°C) on a plate shaker at a setting of 600 rpm ± 50 rpm for 60±5 minutes.

5. Shake out contents of the wells. Wash the wells 4 times as follows:

   5.1. Fill wells with 300 µl Wash Buffer at room temperature taking care not to cross-contaminate adjacent wells. Once all wells have been filled shake out the Wash Buffer.
   5.2. Repeat wash operation (step 1 above) once more.
   5.3. Rotate the plate 180° and repeat the wash operation (step 1 above) a further 2 times.

   After the fourth wash, place plate(s) face down on clean filter paper and allow to drain. To facilitate removal of as much Wash Buffer as possible, it is recommended to hold the plates over absorbent paper and to flick the plate with a finger several times, or alternatively, tap the plates lightly on a hard surface covered with absorbent paper.

   NOTE: Automatic microplate washers may be used provided the number of wash cycles and the delay period between each wash has been optimised to remove background reactions. Ensure that unbound conjugate has been adequately removed from the wells to prevent invalid results. Do not incubate the Wash Buffer on the wells and ensure the resting time of the Wash Buffer is as short as possible i.e. between 18-60 sec. Otherwise, OD signal reduction by too stringent washing may result.

6. Add 100 µl of freshly prepared Conjugate Reagent to wells. The reagent is 1X conjugate diluted in working strength Blue Diluent according to the Reagent Preparation Table (Table 1). Mix thoroughly as in Step 3.

7. Cover each plate and incubate as in Step 4 for 60±5 minutes.

8. Wash wells as in Step 5.

9. Add 100 µl of Enzyme Substrate Solution to wells. Mix thoroughly as in Step 3.

10. Cover each plate with a lid and incubate as in Step 4 for 30 minutes. Protect from direct sunlight. **NOTE:** Commence incubation time as you add substrate to the first well(s).

11. Add 50 µl of Enzyme Stopping Solution to each well, being careful not to cross-contaminate from well to well, then mix by gentle agitation. **NOTE:** The Enzyme Stopping Solution should be added to wells in the same order and at the same speed as the Enzyme Substrate Solution.

12. Read the absorbance of each well within 5 minutes of terminating the reaction using a 450 nm filter with a 620-650 nm reference filter. The absorbance values will then be used to calculate results.

Quality Control (Valid Assay)

The control results must be examined before the sample results can be interpreted. Determine the absorbance of negative and positive controls. Acceptable range of OD values:

- **Negative Bovine IFN-γ Control < 0.130**
  NOTE: The negative controls must not vary by more than 0.040.
- **Positive Bovine IFN-γ Control: 1.20 – 2.00**
  NOTE: The positive control must not deviate by [illegible] from their absorbance

If either of the above criteria is not met, the EIA run is invalid and must be repeated.

Interpretation

1. Compare the absorbance values of the nil antigen, avian and bovine PPD samples for each animal.

Positive = OD Bovine PPD — Nil Antigen ≥ 0.1; and OD Bovine PPD — Avian PPD ≥ 0.1

Negative =OD Bovine PPD — Nil Antigen < 0.1; or OD Bovine PPD — Avian PPD < 0.1

2. Blood plasma collected from cattle having a bovine PPD OD value more than 0.100 above the avian PPD and nil (PBS) antigen, indicates the presence of *Mycobacterium bovis* infection.

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

If the NIL OD value is ≥ 0.3, test interpretation of result is hampered due to a general infection of the animal other than *M. bovis*. Blood collection and testing should be repeated.

If the Pokeweed OD value is ≤ 0.5 test interpretation of result is hampered due to a general decreased viability of cells. Blood collection and testing should be repeated.

Hyper reactors:
If the OD values for both PPD-B and PPD-A exceed 3.5 OD units, the samples have to be diluted with NIL plasma from the same animal, in a ratio of 1 part PPD-B/PPD-A and 2 parts NIL plasma and retested in the EIA. Should the retested samples have OD values which exceed 3.5 units, then the original undiluted samples (PPD-B and PPD-A) need to be diluted in a ratio of 1 part PPD-B/PPD-A and 4 parts NIL plasma and be retested again in the EIA.

As with any biological test, this test may give a false positive or false negative result due to local conditions. A test should be interpreted in the context of all available clinical, historical and epidemiological information relevant to the animal(s) under test. Further confirmatory testing may be required in certain circumstances.

Responsibility for the test interpretation and consequent animal husbandry decisions rests solely on the user, and any consulting veterinarian and appropriate health advisors or authorities.

Prionics AG accepts no responsibility for any loss or damage, howsoever caused, arising from the interpretation of test results.

Limitation of Procedure

False results may occur due to:

1. Incorrect technique.
2. Use of any anticoagulant other than heparin.
3. Excessive levels of circulating IFN-γ.
4. Immunosuppression.
5. Use of contaminated reagents.
6. Other deviations from the recommended test procedure.

Abbreviated Test Procedure

STAGE 1 – WHOLE BLOOD CULTURE

1. Collect blood.
2. Prepare tuberculin working concentration
3. Prepare the cell viability control PWM (recommended)
4. Aliquot heparinized blood.
5. Add antigens.
6. Incubate overnight.
7. Harvest plasmas.
8. Store plasmas (if necessary).

STAGE 2 – BOVINE IFN-γ EIA

1. Reconstitute freeze dried components.
2. Add Green Diluent to required wells.
3. Add test plasmas (simultaneously for each animal) and controls to wells.
4. Incubate for 60±5 minutes.
5. Wash wells.
6. Prepare Conjugate Reagent and add to wells.
7. Incubate for 60±5 minutes.
8. Wash wells.
9. Prepare Enzyme Substrate Solution.
10. Add Enzyme Substrate to wells.
11. Incubate for 30 minutes.
12. Stop reaction.
13. Read results with 450 / 620-650 nm filters.
14. Validate and interpret results.

Additional Layouts for Whole Blood Stimulation and ELISA

Double Well Testing without Pokeweed Control

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	aP A1	NL A2	bP A4	aP A5	NL A6	bP A8	aP A9	NL A10	bP A12	aP A13	NL A14
B	PC	aP A1	NL A2	bP A4	aP A5	NL A6	bP A8	aP A9	NL A10	bP A12	aP A13	NL A14
C	PC	NL A1	bP A3	aP A4	NL A5	bP A7	aP A8	NL A9	bP A11	aP A12	NL A13	bP A15
D	NC	NL A1	bP A3	aP A4	NL A5	bP A7	aP A8	NL A9	bP A11	aP A12	NL A13	bP A15
E	NC	bP A2	aP A3	NL A4	bP A6	aP A7	NL A8	bP A10	aP A11	NL A12	bP A14	aP A15
F	NC	bP A2	aP A3	NL A4	bP A6	aP A7	NL A8	bP A10	aP A11	NL A12	bP A13	aP A15
G	bP A1	aP A2	NL A3	bP A5	aP A6	NL A7	bP A9	aP A10	NL A11	bP A13	aP A14	NL A15
H	bP A1	aP A2	NL A3	bP A5	aP A6	NL A7	bP A9	aP A10	NL A11	bP A13	aP A14	NL A15

Single Well Testing with Pokeweed Control

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	bP A1	bP A3	bP A5	bP A7	bP A9	bP A11	bP A13	bP A15	bP A17	bP A19	bP A21
B	PC	aP A1	aP A3	aP A5	aP A7	aP A9	aP A11	aP A13	aP A15	aP A17	aP A19	aP A21
C	PC	NL A1	NL A3	NL A5	NL A7	NL A9	NL A11	NL A13	NL A15	NL A17	NL A19	NL A21
D	NC	PW A1	PW A3	PW A5	PW A7	PW A9	PW A11	PW A13	PW A15	PW A17	PW A19	PW A21
E	NC	bP A2	NL A4	bP A6	bP A8	bP A10	bP A12	bP A14	bP A16	bP A18	bP A20	bP A22
F	NC	aP A2	NL A4	aP A6	aP A8	aP A10	aP A12	aP A14	aP A16	aP A18	aP A20	aP A22
G	X	NL A2	NL A4	NL A6	NL A8	NL A10	NL A12	NL A14	NL A16	NL A18	NL A20	NL A22
H	X	PW A2	PW A4	PW A6	PW A8	PW A10	PW A12	PW A14	PW A16	PW A18	PW A20	PW A22

Single Well Testing without Pokeweed Control

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	NL A1	aP A4	bP A7	NL A9	aP A12	bP A15	NL A17	aP A20	bP A23	NL A25	aP A28
B	PC	bP A2	NL A4	aP A7	bP A10	NL A12	aP A15	bP A18	NL A20	aP A23	bP A26	NL A28
C	PC	aP A2	bP A5	NL A7	aP A10	bP A13	NL A15	aP A18	bP A21	NL A23	aP A26	bP A29
D	NC	NL A2	aP A5	bP A8	NL A10	aP A13	bP A16	NL A18	aP A21	bP A24	NL A26	aP A29
E	NC	bP A3	NL A5	aP A8	bP A11	NL A13	aP A16	bP A19	NL A21	aP A24	bP A27	NL A29
F	NC	aP A3	bP A6	NL A8	aP A11	bP A14	NL A16	aP A19	bP A22	NL A24	aP A27	bP A30
G	bP A1	NL A3	aP A6	bP A9	NL A11	aP A14	bP A17	NL A19	aP A22	bP A25	NL A27	aP A30
H	aP A1	bP A4	NL A6	aP A9	bP A12	NL A14	aP A17	bP A20	NL A22	aP A25	bP A28	NL A30

NL=Nil Control Antigen (PBS); aP=Avian PPD; bP=Bovine PPD; PC=positive Control; NC=negative Control; PW=Pokeweed Mitogen; A = Animal

Safety Regulations / R&S Statements

Safety Regulations and R&S Statements

National Safety Regulations must be strictly followed.

R&S Statements

Component 1
Microplate coated with antibody to IFN-γ
Hazard code: This product is not classified according to EU regulations.

Component 2
Positive Bovine IFN-γ Control
Hazard code: This product is not classified according to EU regulations.

Component 3
Negative Bovine IFN-γ Control
Hazard code: This product is not classified according to EU regulations.

Component 4
Green Diluent: Sample Diluent Buffer
Hazard code: This product is not classified according to EU regulations.

Component 5
Wash Buffer – 20X Concentrate
Hazard code: This product is not classified according to EU regulations.

Component 6  *Xi ; Sensitising*
Conjugate – 250X Concentrate
Hazard code: Xi Sensitizing
R43: May cause sensitization by skin contact.
S24: Avoid contact with skin.
S37: Wear suitable gloves.

Component 7
Blue Diluent: Conjugate Diluent Buffer
Hazard code: This product is not classified according to EU regulations.

Component 8
Enzyme Substrate Solution
Hazard code: This product is not classified according to EU regulations.

Component 9
Enzyme Stopping Solution  *C Corrosive*
Hazard Code: C Corrosive
R35: Causes severe burns.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

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