DRG® IFN-γ ELISA (EIA-4434)

CE

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Revised 15 Mar. 2011 rm (Vers. 3.1)

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

Immunoenzymetric assay for measurement of human interferon gamma (IFN-γ) in serum and plasma.

2 PRINCIPLES OF THE METHOD

The DRG IFN-γ-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IFN-γ.

Standards and samples react with the capture monoclonal antibody (MAb 1) coated on microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAb 1 – human IFN-γ – MAb 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined calorimetrically by measuring the absorbance, which is proportional to the IFN-γ concentration.

A calibration curve is plotted and IFN-γ concentration in samples is determined by interpolation from the calibration curve. The use of the ELISA reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in a high sensitivity in the low range and in an extended calibration range.

3 REAGENTS PROVIDED

Symbol	Reagents	96 tests Kit	Color Code	Reconstitution
MICROTITERPLATE	Microtiterplate with 96 anti IFN-γ (monoclonal antibodies) coated wells	96 wells	blue	**Ready** for use
Ab HRP	Conjugate: HRP labelled anti-IFN-γ (monoclonal antibodies) in TRIS-Maleate buffer with bovine serum albumin and thymol	1 vial 6 ml	red	**Ready** for use
CAL 0	Zero calibrator in human serum, benzamidin and thymol	3 vial lyophil.	black	**Add** distilled water (see on the label for the exact volume)
CAL N	Calibrator N = 1 to 5 (see exact values on vial labels) in human serum, benzamidin and thymol	5 vials lyophil.	yellow	**Add** 0.5 ml distilled water

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WASH SOLN CONC	Wash Solution (Tris-HCl)	1 vial 10 ml	brown	**Dilute** 200 x with distilled water (use a magnetic stirrer).
CONTROL N	Controls - N = 1 or 2 in human serum, benzamidin and thymol	2 vials lyophil.	silver	**Add** 0.5 ml distilled water
CHROM TMB CONC	Chromogen TMB (Tetramethylbenzydine) in Dimethylformamide	1 vial 1 ml	green	**Dilute** 0.2 ml into 1 vial of substrate buffer
SUB BUF	Substrate buffer: H_2O_2 in acetate / citrate buffer	3 vials 21 ml	white	**Ready** for use
STOP SOLN	Stop Solution: H_2SO_4 1.8N	1 vial 6 ml	black	**Ready** for use

Note: 1. Use the zero standard for sample dilutions.
2. 1 IU of the standard preparation is equivalent to 1 IU of the NIBSC Reference Reagent 87/586.

4 SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. High quality distilled water
2. Pipettes for delivery of: 50 µl, 200 µl, 1 ml and 10 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Horizontal microtiterplate shaker capable of 700 rpm ± 100 rpm
6. Washer for Microtiterplates
7. Microtiterplate reader capable of reading at 450 nm, 490 nm and 650 nm (in case of polychromatic reading) or capable of reading at 450 nm and 650 nm (bichromatic reading)
8. Optional equipment: The ELISA-AID™ necessary to read the plate according to polychromatic reading (see paragraph 10.1.) can be purchased from Robert Maciels Associates, Inc. Mass. 0.2174 USA.

5 REAGENT PREPARATION

A. **Standards**:
Reconstitute the zero standard to the volume specified on the vial label with distilled water and the other standards with 0.5 ml distilled water.

B. **Controls**:
Reconstitute the controls with 0.5 ml distilled water.

C. **Working Wash solution**:
Prepare an adequate volume of Working Wash solution by adding 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

D. **Revelation Solution**:
pipette 0.2 ml of the chromogen TMB into one of the vials of substrate buffer (H_2O_2 in acetate/citrate buffer).
Extemporaneous preparation is recommended.

6 STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, standards and controls are stable for 4 days at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 2 months. Avoid successive freeze thaw cycles.
- The concentrated Wash Solution is stable at room temperature until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the conjugate is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- The freshly prepared revelation solution is stable, before use, for maximum 15 minutes at room temperature and must be discarded afterwards.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

7 SPECIMEN COLLECTION AND PREPARATION

- Serum must be removed as soon as possible from the clot of red cells after clotting and centrifugation, and kept at 4°C. If the samples are not used immediately, they must be kept at -20°C for maximum 2 months, and at -70°C for longer storage (maximum one year).
- Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at room temperature. It is recommended to vortex the samples before use.
- Sampling conditions can affect values, therefore, strict precautions have to be taken during sampling to avoid impurities contained in sampling materials that would stimulate IFN-γ production by blood cells and thus falsely increase plasma IFN-γ values.
- Collection tubes must be pyrogen-free. Plasma can be collected on sterile EDTA and rapidly separated after centrifugation. The use of heparin tubes is discouraged as batches of heparin are often contaminated with pyrogen.

8 PROCEDURE

8.1 Handling notes

Do not use the kit or components beyond expiry date.

Do not mix materials from different kit lots.

Bring all the reagents to room temperature prior to use.

Thoroughly mix all reagents and samples by gentle agitation or swirling.

Perform standards, controls and samples in duplicate. Vertical alignment is recommended.

Use a clean plastic container to prepare the Wash Solution.

In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.

For the dispensing of the Revelation Solution and the Stop Solution avoid pipettes with metal parts.

High precision pipettes or automated pipetting equipment will improve the precision.

Respect the incubation times.

To avoid drift, the time between pipetting of the first standard and the last sample must be limited to the time mentioned in section 12.5 (Time delay).

Prepare a calibration curve for each run, do not use data from previous runs.

The Revelation Solution should be colourless. If a blue colour develops within a few minutes after preparation, this indicates that the reagent is unusable, and must be discarded.

Dispense the Revelation Solution within 15 minutes following the washing of the microtiterplate.

During incubation with Revelation Solution, avoid direct sunlight on the microtiterplate.

8.2 Procedure

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the strips into the holding frame.
3. Pipette 50 µl of each Calibrator, Control and Sample into the appropriate wells.
4. Pipette 50 µl of anti-IFN-γ-HRP conjugate into all the wells.
5. Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
6. Aspirate the liquid from each well.
7. Wash the plate 3 times by:
   Dispensing 0.4 ml of Wash Solution into each well
   Aspirating the content of each well
8. Pipette 200 µl of the freshly prepared Revelation Solution into each well within 15 minutes following the washing step.
9. Incubate the microtiterplate for 15 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
10. Pipette 50 µl of Stop Solution into each well.
11. Read the absorbencies at 450 nm and 490 nm (reference filter 630 nm or 650 nm) within 3 hours and calculate the results as described in section 10.

9 CALCULATION OF RESULTS

9.1 Polychromatic Reading

1. In this case, the ELISA-AID™ software will do the data processing.

2. The plate is first read at 450 nm against a reference filter set at 650 nm (or 630 nm).
3. A second reading is performed at 490 nm against the same reference filter.
4. The ELISA-AID™ Software will drive the reader automatically and will integrate both readings into a polychromatic model. This technique can generate OD's up to 10.
5. The principle of polychromatic data processing is as follows:

   Xi = OD at 450 nm

   Yi = OD at 490 nm

   Using a standard unweighted linear regression, the parameters A & B are calculated : Y = A*X – B

   If Xi < 3 OD units, then X calculated = Xi

   If Xi > 3 OD units, then X calculated = (Yi-B)/A

   A 4-parameter logistic curve fitting is used to build up the calibration curve.

   The IFN-γ concentration in samples is determined by interpolation on the calibration curve.

9.2 Bichromatic Reading

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. On semi-logarithmic or linear graph paper plot the OD values (ordinate) for each standard against the corresponding concentration of IFN-γ (abscissa) and draw a calibration curve through the standard points by connecting the plotted points with straight lines.
4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

10 TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

IFN-γ-ELISA		OD units Polychromatic model
Standard	0 IU/ml	0.03
	1 IU/ml	0.173
	2 IU/ml	0.339
	5 IU/ml	0.700
	10 IU/ml	1.353
	30 IU/ml	3.107

11 REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

12 PRECAUTIONS AND WARNINGS

Safety

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with all reagents, Stop Solution contains H_2SO_4, the chromogen contains TMB in Dimethylformamide, Substrate buffer contains H_2O_2. In case of contact, wash thoroughly with water.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

13 SUMMARY OF THE PROTOCOL

	CALIBRATORS (µl)	SAMPLE(S) CONTROLS (µl)
Calibrators (0-5)	50	-
Samples, Controls	-	50
Anti-IFN-γ -HRP conjugate	50	50
Incubate for 2 hours at room temperature with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash Solution and aspirate.		
Revelation Solution	200	200
Incubate for 15 min at room temperature with continuous shaking at 700 rpm.		
Stop Solution	50	50
Read on a microtiter plate reader and record the absorbance of each well at 450 nm (and 490 nm) versus 630 (or 650 nm)		

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