



# Total IgE IRMA Kit

**REF** 20 IGE125

**Σ** 125



## WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

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## INTENDED USE

The IgE IRMA has been designed for the quantitative *in vitro* diagnostic measurement of Total Immunoglobulin E (IgE) in serum or plasma.

## PRINCIPLES OF THE IRMA

The IRMA is a double antibody immunoradiometric assay system.

The sample is incubated with monoclonal anti-human IgE bound to magnetisable polystyrene particles (solid phase) at 37°C. The <sup>125</sup>I labelled polyclonal goat anti-human IgE is added and there is a second incubation at 37°C. After incubation the resultant "sandwich" is sedimented, decanted and washed to remove unbound <sup>125</sup>I labelled antibody. The tubes containing the sedimented "sandwich" are then counted using a gamma counter. The concentration of the analyte is directly proportional to the bound radioactivity of the sandwich. Counts from the calibrators are plotted and samples are read from the constructed calibrator curve.

## REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 125 tests. The kit and all its components, unopened or opened, should be stored at 2-8°C until the listed expiry dates.

## IgE: Tracer

**1 vial REF # IEI1**  
26 mL containing <sup>125</sup>I labelled anti-IgE (≤13μCi) in BSA PBS buffer, non-immune animal serum and an orange dye. Contains sodium azide, (NaN<sub>3</sub>), 0.1% w/v. Ready to use.

## IgE: Solid Phase

**1 vial REF # IEA1**  
26 mL containing anti-IgE antibody coupled to magnetisable polystyrene particles in BSA PBS buffer. Contains (NaN<sub>3</sub>), 0.1% w/v. Resuspend gently before use.

## Wash Concentrate

**1 vial REF # HW1**  
10 mL of a 15 x concentrated wash solution. Contains (NaN<sub>3</sub>), 1.5% w/v. To be diluted before use.

## IgE: Calibrators

**7 vials REF # IES10**  
5.0 mL in Calibrator 1 and 1.0 mL in Calibrator 2-7, each in horse serum. Contains (NaN<sub>3</sub>), 0.1% w/v. Lyophilized.

## PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

## Specimens and Calibrators

The source material of the calibrators has been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and has been found to be non-reactive for all. However it is recommended that all samples be handled as if capable of transmitting infectious disease.

## Preservatives

The kit contains sodium azide as a preservative. As reagents contain a potentially toxic preservative, care should be taken in handling, to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

## Radioactive Material

The tracer contains radioactive material.

## SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Specimens can be either serum or plasma collected in a manner appropriate for laboratory testing. Serum is preferred, however the anticoagulant heparin or EDTA can be employed without sacrificing accuracy.

Avoid grossly haemolytic, lipaemic and turbid specimens. Specimens can be stored at 2-8°C for up to 48 hours. Specimens held for longer should be stored at or below -20°C. Specimens should not be frozen and thawed repeatedly. Thawed specimens should be checked for flocculent matter and mixed by inversion just prior to testing.

Turbid specimens or specimens containing particulate matter should be centrifuged prior to use.

## MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- \* Distilled or deionised water
- \* Disposable plastic test tubes 12 x 75 mm
- \* Precision pipettes
- \* Repeating pipettes
- \* Timer
- \* Vortex mixer
- \* Water bath (37°C ± 2°C)
- \* Magnetic Racks or Refrigerated centrifuge capable of 1500 x g
- \* Absorbent paper
- \* Gamma counter

## PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use.

## Do not use magnetic stirrer to resuspend solid phase reagent.

Duplicates are recommended. Contamination of reagents will lead to poor performance. A calibrator curve should be run with each assay. Specimens suspected of having concentrations above the top calibrator should be diluted in zero calibrator before assay. All assay steps should be performed without interruption.

Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed. The gamma counter and all pipettes used should be calibrated appropriately before use.

## Washing

The efficiency of the wash step is vital for good precision.

## Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

## ASSAY PROCEDURE

### Preparation of Reagents Wash Solution

Dilute the wash concentrate 1 in 15 with deionised water. The wash solution can be stored at room temperature (20-25°C) for 6 months.

### Calibrators

To reconstitute the lyophilized calibrators, add the volume of deionized water indicated on each vial label. Allow the vials to sit undisturbed until completely dissolved (at least 30 minutes) and then mix by gentle inversion. Exact concentrations determined lot-to-lot are stated on a separate label inside the kit. After reconstitution, the calibrators should be stored at -20°C.

### Protocol

1. Assemble and label test tubes in duplicate according to the number of tests required. Include Total Counts (TC), calibrators, controls and specimens.
2. Pipette 25μL of sample (calibrator, control specimen) in duplicate into the appropriate test tubes.
3. Resuspend the IgE Solid Phase by swirling and repeated inversion of the contents of the bottle until no sediment can be seen on the bottom - do not shake this reagent vigorously.
4. Pipette 200 μL of IgE Solid Phase into all tubes except TC.

5. Vortex tubes gently and then incubate for 30 minutes at 37°C.
6. Pipette 200 µL of IgE Tracer (yellow) into all tubes. Set TC tubes aside.
7. Vortex tubes gently and then incubate for 30 minutes at 37°C.
8. Separation of the sandwich from unbound antibody label may be achieved by using either magnetic separation or centrifugation.
  - A. *Magnetic Separation*
    - a) Place tubes into magnetic separation rack and ensure that all tubes are in contact with the magnetic baseplate. Leave for 5 minutes. Precision can be improved by increasing the time of sedimentation to 10 minutes.
    - b) After separation do not remove rack from magnetic baseplate. Decant the supernatant, and keeping the magnetic baseplate inverted, allow tubes to drain onto absorbent paper for 2 minutes.
  - B. *Centrifugation*
    - a) Centrifuge all tubes for 5 minutes at 1500 x g in a refrigerated centrifuge (4°C). Decant the supernatant and allow tubes to drain onto absorbent paper for 2 minutes.
    - b) Wash the tubes by adding 500 µL wash solution to all tubes. Vortex, centrifuge, decant and blot as above.
9. Count all tubes for one minute using a gamma counter. Record the cpm of each tube.
10. Calculate results.

### CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction.

1. Determine the average cpm for duplicate tubes.
2. Plot the calibrator curve on a semi-log or log-linear graph paper using either of the methods below;

#### Method 1

Use the following formula to calculate %B/T:

$$\%B/T = \frac{\text{cpm (Sample)}}{\text{cpm TC}} \times 100$$

Plot %B/T on the y axis versus the stated concentrations of the calibrators.

#### Method 2

Plot the cpm on the y axis versus the stated concentrations of the calibrators.

3. Read sample values directly off the calibrator curve as kIU/L.

### MODEL CALCULATIONS

ID	Ave cpm	%B/T	IgE kIU/L
TC	118080		
0	126	0.11	
2	520	0.44	
5	1057	0.90	
20	3521	2.98	
100	12944	10.96	
500	28952	24.52	
1500	35566	30.12	
Sample 1	10002	8.47	69.9
Sample 2	16141	13.67	139.2

### CALIBRATION

The calibrators supplied in this kit are calibrated and labelled in kIU/L, referenced to the 2nd International Reference Preparation for IgE 75/502.

### LIMITATIONS

Serum specimens showing gross haemolysis, gross lipaemia, or turbidity may give false results.

Specimens that contain appreciable background radioactivity should not be used. Any suspect specimens should be screened for radioactivity before performing the assay and should be held until the radioactivity has decayed, or a new specimen requested.

### EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. The following reference range was obtained by assaying serum samples from healthy individuals and is given as a guide only: The Total IgE levels were determined using the Bioclone TOTAL IgE IRMA KIT in the serum of 45 Red Cross blood donors who gave no history of allergy.

Geometric mean 32 kIU/L + 1SD = 105 kIU/L

95% were less than 220 kIU/L

### PERFORMANCE CHARACTERISTICS

#### Intra-assay Precision

Sample	n	Mean ± 2SD (kIU/L)	%CV
A	20	36.65 ± 0.73	2.2
B	20	173.60 ± 4.23	2.4
C	20	377.00 ± 11.54	3.1

#### Inter-assay Precision

Sample	n *	Mean ± 2SD (kIU/L)	%CV
A	13	27.9 ± 1.02	3.7
B	13	323.0 ± 19.20	5.9

\* duplicate

#### Specificity

Analyte	Concentration Assayed	Apparent IgE Result (kIU/L)
IgG	3.75 mg/mL	not detectable
IgA	0.29 mg/mL	not detectable
IgM	0.39 mg/mL	not detectable

#### Accuracy

Recovery was calculated by assaying before and after addition of exogenous (x) analyte. 12.5 µL of sample X was added to 12.5 µL of each calibrator.

Sample	IgE (kIU/L) Observed	IgE (kIU/L) Expected	Recovery
			%
x (25 µL)	287.4		
x (12.5 µL) + 12.5 µL	0	139.9	143.7
	2	145.7	144.7
	5	147.9	146.2
	20	150.2	153.7
	100	201.7	193.7
	500	389.3	393.7
	1500	790.8	893.7

#### Dilution

A sample was diluted in zero calibrator, assayed and recovery calculated.

Sample	IgE (kIU/L)	IgE (kIU/L)	% Recovery
	Observed	Expected	
Neat	448.8		
1/2	217.7	224.4	97
1/4	103.9	112.2	93
1/8	52.1	56.1	93
1/16	27.0	28.1	99

#### High-dose Hook Effect

Due to the high-dose hook effect characteristic of the assay, samples greater than 11000 kIU/L may yield aberrant results, less than that of the kits highest calibrator. Those samples should be diluted with the zero calibrator and reassayed.

#### Sensitivity

The sensitivity of the assay is typically <0.32 kIU/L.

The sensitivity is defined as that concentration of analyte which corresponds to the dose response variable (cpm) that is two standard deviations from the mean dose response variable of 10 replicate determinations of the zero calibrator run in three different assays.

#### Interference

No interference with analyte recovery was observed for concentrations of haemoglobin up to 250 mg/dL, bilirubin up to 10 mg/dL and triglycerides up to 970 mg/dL.

### ORDERING INFORMATION

The IgE IRMA is manufactured by:

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### TECHNICAL SERVICE

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