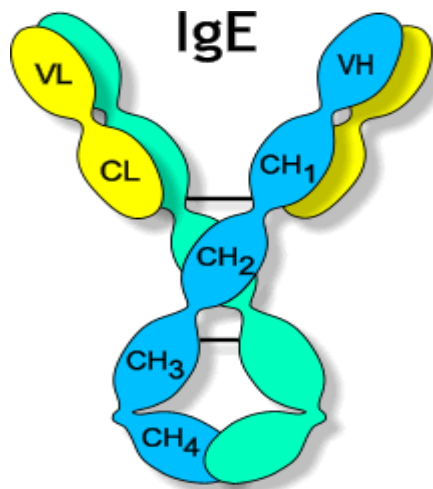


## Total Human IgE ELISA Assay:



### Intended Use:

To quantitate total human Immunoglobulin E (IgE)

### Principle of Procedure:

Solid phase capture sandwich ELISA assay using a microwell format.

### Shelf Life:

The expiration date for the package and each component is stated on the label(s). Store all components at 2-8°C with the exception of the standards, which should be stored frozen.

### Patient and Standard Dilutions:

Patients and standards are diluted in the wells by adding 10uL to 90uL of specimen diluent. The specimen diluent alone is used for a blank.

### Materials Supplied:

Anti-Human IgE coated microwell strips 12x8 with plastic frame – 8 strips  
HRP conjugated mouse monoclonal anti-human IgE – 12mL  
IgE standards (Low, Medium, High) – 200 uL each  
TMB/peroxide substrate color developer – 12mL  
IgE specimen diluent (Specimen Diluent Green II) – 60mL  
Sulfuric acid termination reagent (0.5N) – 12mL  
15 X Wash buffer concentrate – 60mL

\*Quantities are representative of single kits- for double kits multiply amounts by two

### Limitations of the Procedure:

No single assay should be used as the only basis for arriving at a diagnostic conclusion. For invitro diagnostic use.

### Dynamic Range:

0 IU/mL to 400 IU/mL.

- For a higher dynamic range dilute standards and specimen 1:5 with the provided diluent before running assay.

### Reproducibility:

C.V. 6%-10% depending upon the region of the standard curve.

### Assay Procedure:

\*Allow each reagent to reach room temperature before use

1. Add 10uL of specimen or standard to each microwell. .
2. *Add 90ul of specimen diluent to each standard or specimen well.*
3. Incubate at room temperature for 2 hours.
4. Decant and wash each microwell four times with wash buffer (Prediluted 1:15 with reagent grade water.) Firmly grip and pound the microwells upside down on clean, dry, and multiple folded paper towels after the last decantation.
5. Add 100uL of HRP conjugated HRP conjugated mouse monoclonal anti-human IgE to each well.
6. Incubate at room temperature for 2 hours.
7. Decant and wash as in “step 4”.
8. Add 100uL of TMB/Peroxide Substrate and incubate at room temperature for 30 minutes.
9. Terminate the reaction with 100uL of 0.5N sulfuric acid.
10. Zero the microwell reader at 450nm using the specimen diluent zero control well (blank.)
11. Determine the optical density (O.D.) @ 450 nm for each well.
12. Construct a standard curve using the O.D. values obtained for each of the standards.
13. Interpolate (x-axis) the unknowns @ 450 nm from the constructed standard curve (y-axis).

### Typical Standard Curve:

