Mouse Total IgE ELISA

Catalog Number M036023

For the quantitative determination of Total IgE in mouse serum samples.

For research use only.
This product insert must be read in its entirety before using this product.
**PRINCIPLE OF THE ASSAY**

ELISA or Enzyme-linked Immunosorbent Assay is a colorimetric based immunoassay utilizing a monoclonal antibody to mouse IgE bound on the surface of microwells as a capture antibody. HRP-conjugated IgE in the presence of substrate is used as a detection antibody.

**KIT COMPONENTS**

**Total IgE Microplate (Part PL30201)** - The plate contains 12 x 8 strips coated with anti-IgE monoclonal antibody. The strips are ready to use.

**MD-1 Assay Diluent (Part AD30001)** - 1 vial of buffer containing sodium azide.

**Wash Buffer Concentrate (10X) (Part WB30004)** - 1 vial of a 10-fold concentrated buffered surfactant.

**Total IgE Standard (Part ST30199)** - 2500 ng/mL of IgE in a protein buffer.

**Conjugate Concentrate (Part GP30200)** - 1 vial of a 100-fold concentrated goat anti-mouse (GAM)-HRP conjugated to IgE in a stabilizing buffer.

**HRP Diluent (Part SD30003)** - 1 vial of a protein buffer.

**Substrate (Part TM30008)** - 1 vial of a TMB solution ready to use. Protect from light

**Stop Solution (Part SS30007)** - 1 vial of 1.0 N Sulfuric acid. *Warning: Wear eye, hand, face, and clothing protection when using this material.*

**Plate Sealer** - 2 Adhesive strips.
## STORAgE

<table>
<thead>
<tr>
<th>Unopened kit</th>
<th>The Conjugate Concentrate and the Standard need to be stored at -20 º C. All other reagents are stored at 2 - 4º C. Do not use past the kit expiration date.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Diluent</td>
<td>Store at 2 - 8º C for up to 30 days.</td>
</tr>
<tr>
<td>Substrate</td>
<td></td>
</tr>
<tr>
<td>Wash Buffer 1X</td>
<td></td>
</tr>
<tr>
<td>Stop Solution</td>
<td></td>
</tr>
<tr>
<td>Conjugate Concentrate</td>
<td>Store at -20º C for up to 30 days. Avoid repeated freeze-thaw cycles.</td>
</tr>
<tr>
<td>Diluted Conjugate</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td></td>
</tr>
<tr>
<td>Microplate Wells</td>
<td>Return unused wells to the foil pouch containing the desiccant and seal. Store at 2 - 8º C for up to 30 days.</td>
</tr>
</tbody>
</table>

## SUPPLIES REQUIRED BUT NOT PROVIDED

- Pipettes or pipetting equipment with disposable polypropylene tips
- Glass measuring cylinders
- Distilled or deionized water
- Horizontal orbital microplate reader
- Squirt bottle or automated microplate washer
- Microplate reader capable of measuring at 450 nm
- Orbital shaker
**PRECAUTIONS**

Stop Solution consists of diluted sulfuric acid. Wear eye, hand, face, and clothing protection when using these materials. Avoid contact with skin and eyes. In case of contact wash immediately with water. All chemicals should be considered as being potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.

The Assay Diluent contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

- For research use only. Not for internal or external use in humans or animals.
- This kit contains no material of human origin.
- For the handling of blood, (serum), we recommend that precautions should be observed.
- Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/ national guidelines on laboratory safety procedures.

**CRITICAL PARAMETERS**

- Allow samples and all reagents to equilibrate to room temperature (22 - 25 °C) prior to performing the assay. This is especially important for the TMB Substrate.
- Adhere to recommended incubation temperatures in the protocol as variations may cause inconsistent or poor assay results.
- It is essential that all wells are washed thoroughly and uniformly. When washing is done by hand, use a squeeze bottle and ensure that all wells are completely filled and emptied at each step.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.
- Mix all reagents thoroughly prior to use, but avoid foaming!
- Keep the wells sealed with the plate sealer except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. Other sample types may be tested and validated by the user.
SAMPLE COLLECTION & STORAGE

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C.

SAMPLE PREPARATION

Serum samples require at least a 100-fold dilution into Assay Diluent.

REAGENT PREPARATION

Bring all reagents to room temperature (22 - 25 °C) before use. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.

Wash Buffer (1X) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL of deionized water to prepare 500 mL of Wash Buffer (1X). Store for up to 30 days at 2 - 8 ºC.

Conjugate (1X) - Dilute 110 µL of Conjugate Concentrate into 11 mL of HRP diluent. Store diluted conjugate for up to 30 days at -20 ºC.

Standards - Label 6 standard tubes as shown below. Pipette 400 µL Assay Diluent into the 500 ng/mL standard tube and 250 µL Assay Diluent into the remaining tubes. Use the 2500 ng/mL standard to produce a dilution series (see below). The 500 ng/mL standard serves as the high standard and Assay Diluent serves as the zero (0 ng/mL) standard.
ASSAY PROTOCOL

Read the entire protocol before beginning the assay. It is recommended that all standards and samples be assayed in duplicate. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use. Note: Reagents and samples may require specific handling temperatures and need preparation prior to the assay. See the Reagent and Sample Preparation sections before proceeding.

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microplate strips from the plate frame and return them to the foil pouch containing the desiccant pack.
3. Pipette 100 µL of Standard or sample in duplicate into the wells using a clean pipette tip for each standard or sample. Cover with the plate sealer provided and incubate for 1 hour at room temperature (22 - 25 °C) on an orbital shaker (600 rpms).
4. Aspirate and wash the wells 4 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
5. Add 100 µL of Conjugate (1X) into each well. Cover with the plate sealer provided and incubate for 1 hour at room temperature on the shaker.
6. Aspirate and wash the wells 4 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
7. Add 100 µL Substrate to each well and incubate for 15 minutes at room temperature. Protect from light.
8. Stop the reaction by adding 100 µL of Stop Solution to each well. Gently tap the side of the plate to ensure thorough mixing.
9. Read the plate at 450 nm.
SUMMARY

Prepare reagents and samples as previously described.

↓

Pipette 100 µL Standard or diluted sample in duplicate into the wells. Incubate 1 hr at RT (22 - 25 °C) on shaker.

↓

Aspirate and wash 4 times.

↓

Add 100 µL of Conjugate (1X) to each well. Incubate 1 hour at RT on shaker.

↓

Aspirate and wash 4 times.

↓

Add 100 µL of Substrate to each well. Incubate 15 min. at RT. Protect from light.

↓

Add 100 µL of Stop Solution to each well. Read at 450 nm
**CALCULATION OF RESULTS**

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Total IgE concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

This standard curve is provided for demonstration only. A standard curve should be generated with each set of samples assayed.

![Standard Curve](image)

**PERFORMANCE CHARACTERISTICS**

**Sensitivity**

Sensitivity is defined as the minimal detectable dose determined by adding two standard deviations of the mean optical density value for twenty replicates of the zero standard and calculating the corresponding concentration. The sensitivity of the Total IgE ELISA is typically less than 2.0 ng/mL.
Reproducibility

**Intra-assay Precision** (Precision within an assay) - The intra-assay precision was measured by assaying three control samples 15-20 times on one plate.

**Inter-assay Precision** (Precision between assays) - The inter-assay precision was assessed by repeated measurements of three control samples in 20 successive assays with multiple users.

<table>
<thead>
<tr>
<th>Control</th>
<th>Intra-assay Precision</th>
<th>Inter-assay Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mean (ng/mL)</td>
<td>21.8</td>
<td>79.3</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1.90</td>
<td>5.80</td>
</tr>
<tr>
<td>CV (%)</td>
<td>8.7</td>
<td>7.4</td>
</tr>
</tbody>
</table>

LINEARITY

Natural mouse IgE samples were diluted 100-fold in Assay Diluent and serially diluted down to 1:16.

<table>
<thead>
<tr>
<th>Species</th>
<th>Average</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>102%</td>
<td>84 - 121%</td>
</tr>
</tbody>
</table>

SPECIFICITY

Mouse IgG was evaluated in the assay at different concentrations. The cross-reactivity of IgG is less than 0.01%.
# TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Absorbance</td>
<td>· Check reagents for proper storage</td>
</tr>
<tr>
<td></td>
<td>· Control expiration date.</td>
</tr>
<tr>
<td></td>
<td>· Check preparation of reagents.</td>
</tr>
<tr>
<td></td>
<td>· Control incubation times and temperature.</td>
</tr>
<tr>
<td></td>
<td>· Check reader wavelength.</td>
</tr>
<tr>
<td>High Absorbance/high zero standard value</td>
<td>· Check preparation of reagents.</td>
</tr>
<tr>
<td></td>
<td>· Control incubation times and temperature.</td>
</tr>
<tr>
<td></td>
<td>· Equilibrate ELISA reagents to room temperature (22 - 25 °C).</td>
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<tr>
<td></td>
<td>· Ensure that every well of the ELISA plate is completely filled and emptied at every wash step.</td>
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<td></td>
<td>· Check that plates are blotted on tissue paper after washing.</td>
</tr>
<tr>
<td>Flat curve/poor reproducibility</td>
<td>· Check reagents for proper storage.</td>
</tr>
<tr>
<td></td>
<td>· Control expiration date.</td>
</tr>
<tr>
<td></td>
<td>· Check preparation of working standards.</td>
</tr>
<tr>
<td></td>
<td>· Check incubation times and temperatures.</td>
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<tr>
<td></td>
<td>· Use separate reservoirs for pipetting different solutions with multichanneled pipettes. Always use new pipette tips.</td>
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<tr>
<td></td>
<td>· Check pipette calibration.</td>
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<tr>
<td></td>
<td>· Ensure efficient washing procedure.</td>
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