IgE Human Uncoated ELISA Kit with Plates

Enzyme-linked immunosorbent assay for quantitative detection of human IgE

Catalog Number 88-50610

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Standard curve of IgE Human Uncoated ELISA Kit with Plates

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Product information

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Contents</th>
<th>IgE Human Uncoated ELISA Kit with Plates</th>
</tr>
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<tbody>
<tr>
<td>REF</td>
<td>Catalog number</td>
<td>88-50610</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>7.8 ng/ml</td>
</tr>
<tr>
<td></td>
<td>Standard curve range</td>
<td>1,000–7.8 ng/ml</td>
</tr>
<tr>
<td></td>
<td>Temperature limitation</td>
<td>Store at 2–8°C</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
<td>Refer to vial</td>
</tr>
<tr>
<td></td>
<td>Use by</td>
<td>Refer to box label</td>
</tr>
<tr>
<td></td>
<td>Caution</td>
<td>Contains preservatives</td>
</tr>
</tbody>
</table>

Description

This IgE Human Uncoated ELISA Kit with Plates contains the necessary reagents, standards, buffers, and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for accurate and precise measurement of human IgE protein levels from samples including serum and plasma.

Components of 2-plate format (2x96 tests)

Capture Antibody: Pre-titrated, purified anti-human IgE monoclonal antibody
1 vial (100 µL) Capture Antibody Concentrate (250x)

Detection Antibody: Pre-titrated, HRP-conjugated anti-human IgE monoclonal antibody
1 vial (100 µL) Detection Antibody Concentrate (250x)

Standard: Recombinant human IgE for generating standard curve and calibrating samples
2 vials human IgE Standard (lyophilized): 1,000 ng/ml upon reconstitution

Coating Buffer: 1 vial (2.5 ml) Phosphate Buffered Saline Concentrate (PBS) 10x

Assay Buffer A: 1 bottle (10 ml) Assay Buffer A Concentrate 20x (PBS with 1% Tween™ 20 and 10% BSA)

Substrate Solution: Tetramethylbenzidine (TMB) Substrate Solution 1 bottle (25 ml)
2 96-well plates

Components of 10-plate format (10x96 tests)

Capture Antibody: Pre-titrated, purified anti-human IgE monoclonal antibody
1 vial (500 µL) Capture Antibody Concentrate (250x)

Detection Antibody: Pre-titrated, HRP-conjugated anti-human IgE monoclonal antibody
1 vial (500 µL) Detection Antibody Concentrate (250x)

Standard: Recombinant human IgE for generating standard curve and calibrating samples
10 vials human IgE Standard (lyophilized): 1,000 ng/ml upon reconstitution

Coating Buffer: 1 vial (12 ml) Phosphate Buffered Saline Concentrate (PBS) 10x

Assay Buffer A: 1 bottle (50 ml) Assay Buffer A Concentrate 20x (PBS with 1% Tween™ 20 and 10% BSA)

Substrate Solution: Tetramethylbenzidine (TMB) Substrate Solution 1 bottle (120 ml)
10 96-well plates (included with product catalog numbers ending in suffixes -76, -86)

Other materials needed

- Buffers
  - Wash Buffer: 1x PBS, 0.05% Tween™ 20 or eBioscience™ Wash Buffer (20x) Cat. No. BMS408.0500
  - Stop Solution: 1 M H3PO4 or 2 N H2SO4 or eBioscience™ Stop Solution Cat. No. BMS409.0100
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning™ Costar™ 9018)
Note: The use of ELISA plates that are not high-affinity protein-binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning “Costar” 9018 or Nunc™ MaxiSorp™ 96-well plates provided or suggested.

- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

Note: To ensure optimal results from using this kit, use only the components included in the set. Exchanging of components is not recommended because a change in performance may occur.

### Stability
This kit is guaranteed to perform as defined if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents. Expiration date is indicated on the box label.

### Storage instructions for kit reagents
Store at 2-8°C.

### Reagent preparation

**Note:** The use of ELISA plates that are not high-affinity protein-binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning “Costar” 9018 or Nunc™ MaxiSorp™ 96-well plates provided or suggested.

1. **Coating Buffer** (1x)
   - Make a 1:10 dilution of PBS (10x) in deionized water.
2. **Blocking Buffer** (2x)
   - Make a 1:10 dilution of Assay Buffer A Concentrate (20x) in deionized water.
3. **Assay Buffer A** (1x)
   - Make a 1:20 dilution of Assay Buffer A Concentrate (20x) in deionized water.
4. **Capture Antibody**
   - Dilute capture antibody (250x) 1:250 in Coating Buffer (1x).
5. **Standard**
   - Reconstitute human IgE standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Allow the standard to reconstitute for 10-30 minutes. Swirl or mix gently to ensure complete and homogeneous solubilization (concentration of reconstituted standard = 1,000 ng/ml). Mix well prior to making dilutions. The standard has to be used immediately after reconstitution and cannot be stored.
6. **Detection Antibody**
   - Dilute detection antibody (250x) 1:250 in Assay Buffer A (1x).

### Experimental procedure

**Note:** In case of incubation without shaking, the obtained O.D. values may be decreased. Nevertheless the results are still valid.

**Note:** Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

**Note:** If instructions of this protocol have been followed samples have been diluted 1:10, the concentration read from the standard curve must be multiplied by the dilution factor (x10).

1. **Coat Corning “Costar” 9018 ELISA plate with 100 µL/well of capture antibody in Coating Buffer (dilute as noted in point 1 of Reagent preparation). Seal the plate and incubate overnight at 4°C.
2. **Prepare the Blocking Buffer** (see point 2 in Reagent preparation).
## ELISA troubleshooting guide

<table>
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<tr>
<th>Problem</th>
<th>Possibility</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>High background</td>
<td>Improper and inefficient washing.</td>
<td>Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed.</td>
</tr>
<tr>
<td></td>
<td>Cross contamination from other specimens or positive controls.</td>
<td>Repeat ELISA, be careful when washing and pipetting.</td>
</tr>
<tr>
<td></td>
<td>Contaminated substrate.</td>
<td>Substrate should be colorless.</td>
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<tr>
<td></td>
<td>Incorrect dilutions, e.g., conjugate concentration was too high.</td>
<td>Repeat test using correct dilutions; check with manufacturer.</td>
</tr>
<tr>
<td>No signal</td>
<td>Improper, low protein binding capacity plates were used.</td>
<td>Repeat ELISA, using recommended high binding capacity plates.</td>
</tr>
<tr>
<td></td>
<td>Wrong substrate was used.</td>
<td>Repeat ELISA, use the correct substrate.</td>
</tr>
<tr>
<td></td>
<td>Enzyme inhibitor present in buffers, e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity.</td>
<td>Repeat ELISA, make sure your system contains no enzyme inhibitor.</td>
</tr>
<tr>
<td>Very weak signal</td>
<td>Improper and inefficient washing.</td>
<td>Make sure washing procedure is done correctly.</td>
</tr>
<tr>
<td></td>
<td>Incorrect dilutions of standard.</td>
<td>Follow recommendations of standard handling exactly as written on the certificate of analysis.</td>
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<tr>
<td></td>
<td>Insufficient incubation time.</td>
<td>Repeat ELISA, follow the protocol carefully for each step’s incubation time.</td>
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<tr>
<td></td>
<td>Incorrect storage of reagents.</td>
<td>Store reagents in the correct temperature, avoid freeze and thaw, avoid using the frost free freezer.</td>
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<tr>
<td></td>
<td>Wrong filter in ELISA reader was used.</td>
<td>Use correct wavelength setting.</td>
</tr>
<tr>
<td></td>
<td>Wrong plate used.</td>
<td>Use the recommended Corning™ Costar™ 9018 or Nunc™ MaxiSorp™ flat bottom 96-well plates.</td>
</tr>
<tr>
<td>Variation among replicates</td>
<td>Improper and inefficient washing.</td>
<td>Make sure washing procedure is done correctly; see certificate of analysis.</td>
</tr>
<tr>
<td></td>
<td>Poor mixing of samples.</td>
<td>Mix samples and reagents gently and equilibrate to proper temperature.</td>
</tr>
<tr>
<td></td>
<td>Plates not clean.</td>
<td>Plates should be wiped on bottom before measuring absorbance.</td>
</tr>
<tr>
<td></td>
<td>Improper, low binding capacity plates were used.</td>
<td>Use recommended high binding capacity plates.</td>
</tr>
<tr>
<td></td>
<td>Reagents have expired.</td>
<td>Do not use if past expiration date.</td>
</tr>
<tr>
<td>Variation of kit performance</td>
<td>Different buffers, plates.</td>
<td>Use eBioscience™ buffers, plates, and kit components available.</td>
</tr>
<tr>
<td></td>
<td>Handling can strongly affect kit performance.</td>
<td></td>
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- Product documentation, including:
  - User guides, manuals, and protocols

- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)

**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

### Limited product warranty


Manufacturer’s address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.

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