# Human IgE Uncoated ELISA Kit

## Enzyme-linked immunosorbent assay for quantitative detection of human IgE

Catalog Number 88-50610

Pub. No. MAN0016742 Rev. D (40)

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

## Product information

| Symbol | Contents | Human IgE Uncoated ELISA Kit |
|---|---|---|
| REF | Catalog number | 88-50610 |
| — | Sensitivity | 7.8 ng/mL |
| — | Standard curve range | 7.8–500 ng/mL |
| | Temperature limit | Store at 2–8°C |
| LOT | Batch code | See vial |
| | Use by | See box label |
| | Caution | Contains preservatives |

### Description

This Human IgE Uncoated ELISA Kit 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 (2 × 96 tests)

- **Capture Antibody:**
  Pretitrated, purified anti-human IgE monoclonal antibody
  1 vial (100 µL) Capture Antibody Concentrate (250X)
- **Detection Antibody:**
  Pretitrated, HRP-conjugated anti-human IgE monoclonal antibody
  1 vial (100 µL) Detection Antibody Concentrate (250X)
- **Standard:**
  Human IgE Standard for generating standard curve and calibrating samples
  1 vial Human IgE Standard (20X) (lyophilized): 10 µg/mL upon reconstitution
- **Coating Buffer:**
  1 vial (2.5 mL) Phosphate Buffered Saline Concentrate (PBS, 10X)
- **Assay Buffer:**
  1 bottle (10 mL) Assay Buffer Concentrate 20X (PBS with 1% Tween™-20 and 10% BSA)
- **Substrate Solution:**
  Tetramethylbenzidine (TMB) Substrate Solution; 1 bottle (20 mL)
- **96-well plates:**
  2 plates

## Components of 10-plate format (10 × 96 tests)

- **Capture Antibody:**
  Pretitrated, purified anti-human IgE monoclonal antibody
  1 vial (500 µL) Capture Antibody Concentrate (250X)
- **Detection Antibody:**
  Pretitrated, HRP-conjugated anti-human IgE monoclonal antibody
  1 vial (500 µL) Detection Antibody Concentrate (250X)
- **Standard:**
  Human IgE Standard for generating standard curve and calibrating samples
  1 vial Human IgE Standard (20X) (lyophilized): 10 µg/mL upon reconstitution
- **Coating Buffer:**
  1 vial (12 mL) Phosphate Buffered Saline Concentrate (PBS, 10X)
- **Assay Buffer:**
  1 bottle (50 mL) Assay Buffer Concentrate 20X (PBS with 1% Tween™-20 and 10% BSA)
- **Substrate Solution:**
  Tetramethylbenzidine (TMB) Substrate Solution; 1 bottle (100 mL)
- **96-well plates:**
  10 plates (**only** included with product catalog numbers ending in suffixes –76 and –86)

## Required materials not supplied

- Buffers
  - Wash Buffer: 1X PBS, 0.05% Tween™–20 or ELISA Wash Buffer Powder Cat. No. 00-0400-46
  - Stop Solution: 1 M $H_3PO_4$ or 2 N $H_2SO_4$ or Stop Solution Cat. No: SS03 or SS04
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Nunc™ MaxiSorp™)

  **Note:** The use of ELISA plates that are not high-affinity protein-binding plates will result in suboptimal performance, for example, low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Nunc™ MaxiSorp™ 96-well plates provided or suggested.
- Microplate shaker
- 96-well ELISA plate reader (microplate spectrophotometer)
- Plate sealer
- *(Optional)* ELISA plate washer

## Stability

This kit is guaranteed to perform as defined if stored and handled according to instructions of this manual. Expiration date is indicated on the box label.

## Storage instructions for kit reagents

Store undiluted original kit reagents at 2–8°C unless otherwise described.

## Procedural guidelines

- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date.
- Do not expose kit reagents to strong light during storage or incubation.
- To avoid microbial contamination, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.

## Before you begin

- Equilibrate the buffer concentrates to room temperature (18–25°C), then dilute before use.
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.

## Prepare reagents

**IMPORTANT!**

- Diluted Wash Buffer and Coating Buffer are stable for 30 days if stored at 2–25°C.
- Assay Diluent (1X) is stable for one week if stored at 2–8°C.
- Enzyme and Detection Antibody should be diluted 30 minutes before use.
- After dilution return unused stock of Detection Antibody to the refrigerator.

1. **Prepare Coating Buffer (1X)**

   Make a 1:10 dilution of PBS (10X) in deionized water.

   **Table 1 Dilution for 1 plate of Coating Buffer (1X)**

| Number of plates - 96 wells | Coating Buffer concentrate (10X) | Distilled water |
|---|---|---|
| 1 | 1.2 mL | 10.8 mL |

2. **Prepare Blocking Buffer (2X)**

   Make a 1:10 dilution of Assay Buffer Concentrate (20X) in deionized water.

   **Table 2 Dilution for 1 plate of Blocking Buffer (2X)**

| Number of plates - 96 wells | Assay Buffer Concentrate (20X) | Distilled water |
|---|---|---|
| 1 | 1.2 mL | 10.8 mL |

3. **Prepare Assay Buffer (1X)**

   Make a 1:20 dilution of Assay Buffer Concentrate (20X) in deionized water.

4. **Prepare Capture Antibody Solution (1X)**

   Dilute Capture Antibody Concentrate (250X) 1:250 in Coating Buffer (1X).

   **Table 3 Dilution for 1 plate of Capture Antibody Solution (1X)**

| Number of plates - 96 wells | Capture Antibody Concentrate (250X) | Coating Buffer (1X) |
|---|---|---|
| 1 | 0.048 mL | 11.952 mL |

5. **Prepare Standard**

   a. Reconstitute lyophilized Standard (20X) by addition of distilled water.

   Reconstitution volume is stated on the label of the standard vial.

   b. Allow the lyophilized standard to reconstitute for 10–30 minutes.

   c. Swirl or mix gently to ensure complete and homogeneous solubilization (concentration of reconstituted standard = 10 µg/mL).

   d. The reconstituted standard must be stored in single use aliquots at –20°C.

   **Note:**
   - When stored properly, the reconstituted standard is stable and usable for up to 6 months.

- Avoid repeated freeze-thaw cycles.

e. The reconstituted concentrated thawed single use (20X) standard aliquot must be diluted 1:10 in Assay Buffer (1X) in a clean plastic tube.

Shake gently to mix. (Concentration of diluted standard = 1000 ng/mL = 2 × S1).

**IMPORTANT!** Any remaining diluted standard must be discarded after 1 hour of use.

**Table 4 Dilution for 2 × S1 Standard concentration**

| Number of Standards | Single use Standard aliquot (20X) | Assay Buffer (1X) |
|---|---|---|
| 1 | 25 µL | 225 µL |

**Note:** Table 4 is an example of a 1:10 dilution. Adjust the ratio as needed.

6. **Prepare Detection Antibody Solution**

Dilute Detection Antibody Concentrate (250X) 1:250 in Assay Buffer (1X).

**Table 5 Dilution for 1 plate of Detection Antibody Solution (1X)**

| Number of plates - 96 wells | Detection Antibody Concentrate (250X) | Assay Buffer (1X) |
|---|---|---|
| 1 | 0.048 mL | 11.952 mL |

## Perform ELISA assay

**Note:**

- Shaking is necessary for all incubation steps to obtain optimal test performance values unless otherwise noted.
- In case of incubation without shaking, the obtained O.D. values may be decreased. Nevertheless the results are still valid.
- Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.
- 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 (× 10).

### 1 Coat and block the plate

1. Coat the ELISA plate with 100 µL/well of Capture Antibody Solution (1X) (dilute as noted in “Prepare reagents” on page 2). Seal the plate and incubate overnight at 4°C without shaking.
2. Prepare the Blocking Buffer (2X) (see “Prepare reagents” on page 2).
3. Aspirate wells and wash twice with 400 µL/well Wash Buffer.

   Allowing time for soaking (~1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
4. Block wells with 250 µL of Blocking Buffer (2X). Incubate at room temperature for 2 hours (or overnight 4°C) without shaking.

### 2 Add Standards and Samples

1. Prepare the diluted Standard (2 × S1) (see “Prepare reagents” on page 2).
2. Aspirate/wash with Wash Buffer according to step 1.3. Repeat for a total of 2 washes.
3. Perform 2-fold serial dilutions of the standards with Assay Buffer (1X) to make the standard curve.
   a. Add 100 µL of Assay Buffer (1X) to all standard wells. Add 100 µL prepared diluted 2 × S1 concentrated standard in duplicate into wells A1 and A2.
   b. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1=500 ng/mL) and transfer 100 µL to wells B1 and B2, respectively.
   c. Do not scratch surface of the microwells. Continue this procedure 5 times.

## 2 *(continued)*

d. Discard 100 µL from the last standard well to align the volume with the other standard wells.

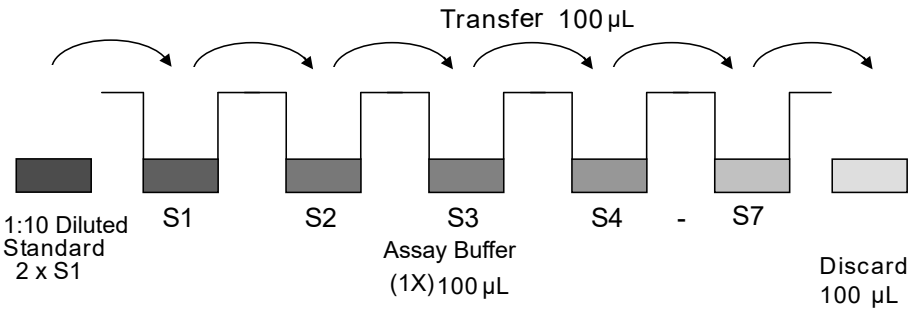


**Figure 1 Standard dilutions on the microwell plate**

**Table 6 Example of the arrangement of blanks, standards, and samples in the microwell strips**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Standard | | Samples | | | | | | | | | |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 7 | 7 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | Blank | Blank | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |

4. Add 100 µL/well of Assay Buffer (1X) to the blank wells.
5. Add 90 µL/well of Assay Buffer (1X) to the sample wells.
6. Add 10 µL/well of your samples to the appropriate wells.
7. Seal the plate and incubate on a microplate shaker at 400 rpm for 2 hours.

## 3 Add Detection Antibody Solution (1X)

1. Prepare the Detection Antibody Solution (1X) (see “Prepare reagents” on page 2).
2. Aspirate/wash with Wash Buffer according to step 1.3. Repeat for a total of 4 washes.
3. Add 100 µL/well diluted Detection Antibody Solution (1X) to all wells used.
4. Seal the plate and incubate on a microplate shaker at 400 rpm for 1 hour.

## 4 Add Substrate Solution

1. Aspirate/wash with Wash Buffer according to step 1.3. Repeat for a total of 4 washes.
2. Add 100 µL/well of Substrate Solution to all wells used. Incubate plate at room temperature for 15–30 minutes, until S1 has developed a dark blue color.

## 5 Add Stop Solution

1. Add 100 µL of Stop Solution to each well.
2. Read plate at 450 nm. If wavelength substraction is available, substract the values of 620 nm from those of 450 nm and analyze data.

## Troubleshooting and FAQs

Visit our online FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated with new information, guidance, and data.

- For troubleshooting information and FAQs for this product: **https://www.thermofisher.com/trizolfaqs**
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  - 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

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

**Revision history:** Pub. No. MAN0016742 D (40)

| Revision | Date | Description |
|---|---|---|
| D (40) | 12 January 2026 | • Removed Standard Curve.<br>• Updated Standard Concentration to 20X.<br>• Minor updates were made throughout for consistency of style and terminology.<br>• Updated Prepare reagents section. |
| C (40) | 4 August 2021 | Baseline document |