invitrogen PRODUCT INFORMATION SHEET

Mouse IgE Uncoated ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of mouse IgE

Catalog Number 88-50460

Pub. No. MAN0016741 Rev. B (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	Mouse IgE Uncoated ELISA Kit
REF	Catalog number	88-50460
—	Sensitivity	4 ng/mL
—	Standard curve range	4–250 ng/mL
	Temperature limit	Store at 2–8°C
LOT	Batch code	See vial
	Use by	See box label
	Caution	Contains preservatives

Description

This Mouse 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 Mouse IgE protein levels from samples including serum, plasma, and supernatants from cell cultures.

Components of 2-plate format (2 × 96 tests)

- **Capture Antibody**
  Pretitrated, purified anti-mouse IgE monoclonal antibody
  1 vial (100 µL) Capture Antibody Concentrate (250X)
- **Detection Antibody**
  Pretitrated, HRP-conjugated anti-mouse IgE monoclonal antibody
  1 vial (100 µL) Detection Antibody Concentrate (250X)
- **Standard:**
  Mouse IgE Standard for generating standard curve and calibrating samples
  1 vial Mouse IgE Standard (20X) (lyophilized): 5000 ng/mL upon reconstitution
- **Coating Buffer**
  1 vial (2.5 mL) Phosphate Buffered Saline Concentrate (PBS, 10X)
- **Assay Buffer:**
  2 bottles (10 mL) Assay Buffer Concentrate 20X (PBS with 1% Tween™-20 and 10% BSA)
- **Detection Enzyme:**
  Pre-titrated Streptavidin-HRP (400X): 1 vial (70 µL)
- **Substrate Solution**
  Tetramethylbenzidine (TMB) Substrate Solution; 1 bottle (20 mL)
- **96-well plates**
  2 plates

Components of 10-plate format (10 × 96 plates)

- **Capture Antibody:**
  Pretitrated, purified anti-mouse IgE monoclonal antibody
  1 vial (500 µL) Capture Antibody Concentrate (250X)
- **Detection Antibody:**
  Pretitrated, HRP-conjugated anti-mouse IgE monoclonal antibody
  1 vial (500 µL) Detection Antibody Concentrate (250X)
- **Standard:**
  Mouse IgE Standard for generating standard curve and calibrating samples
  1 vial Mouse IgE Standard (20X) (lyophilized): 5000 ng/mL upon reconstitution
- **Coating Buffer**
  1 vial (12 mL) Phosphate Buffered Saline Concentrate (PBS, 10X)
- **Assay Buffer:**
  2 bottles (50 mL) Assay Buffer Concentrate 20X (PBS with 1% Tween™-20 and 10% BSA)
- **Detection Enzyme:**
  Pre-titrated Streptavidin-HRP (400X): 1 vial (250 µL)
- **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 = 5000 ng/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 = 500 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

7. **Prepare Enzyme Solution**

Dilute Streptavidin-HRP (400X) 1:400 in Assay Buffer (1X), then mix well.

Table 6 Dilution for 1 plate of Enzyme Solution(1X)

Number of plates - 96 wells	Streptavidin-HRP (400X)	Assay Buffer (1X)
1	0.03 mL	11.97 mL

8. **Prepare Samples**

You may need to further dilute your samples if the analyte concentration exceeds the assay upper limit of quantitation (ULOQ). When preparing dilution of samples use Assay Buffer (1X).

For serum and plasma samples our recommended starting dilution is 25-fold in Assay Buffer (1X).

This dilution was obtained by using samples from CD-1 mice.

Note: The dilution indicated above is only our recommendation.

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, serum and plasma samples have been prediluted 1:25 and then diluted 1:2 on the plate. The concentration read from the standard curve must be multiplied by the dilution factor (× 50).

1 Coat and block the plate

1. Coat the ELISA plate with 100 µL/well of Capture Antibody Solution (1X) (dilute as noted in). 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.

2 *(continued)*

b. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1=250 ng/mL) and transfer 100 µL to wells B1 and B2, respectively.

c. Do not scratch surface of the microwells. Continue this procedure five times.

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

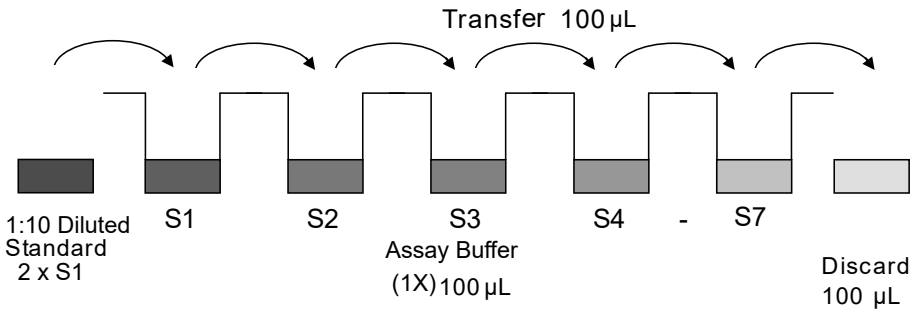

Figure 1 Standard dilutions on the microwell plate

Table 7 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 50 µL/well of Assay Buffer (1X) to the sample wells.

6. Add 50 µL/well of your prediluted serum or plasma samples to the appropriate wells, prediluting them at least 25-fold (1:25) in Assay Buffer (1X). Dilution of other biological samples needs to be determined empirically.

7. Seal the plate and incubate on a microplate shaker at 400 rpm for 2 hours.

3 Add Detection Antibody Solution (1X)

1. Aspirate/wash with Wash Buffer according to step 1.3. Repeat for a total of 4 washes.

2. Prepare the Detection Antibody Solution (1X) (see “Prepare reagents” on page 2).

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 Enzyme Solution (1X)**	1. Prepare the Enzyme Solution (1X) (see Prepare Detection Enzyme on page 3). 2. Add 100 µL of diluted Enzyme Solution (1X) to all wells used. 3. Seal the plate and incubate on a microplate shaker at 400 rpm for 30 minutes at room temperature.
5	**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.
6	**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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  - 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

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

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

Revision history: Pub. No. MAN0016741 B (40)

Revision	Date	Description
B (40)	25 November 2025	• Removed Standard Curve. • Updated Standard Concentration to 20X. • Minor updates were made throughout for consistency of style and terminology. • Updated dilution method of samples. • Updated Prepare reagents section.
A.0 (10)	8 August 2018	Initial release.

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

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