**IgG3 Mouse Uncoated ELISA Kit with Plates**

Enzyme-linked Immunosorbent Assay for quantitative detection of mouse IgG3

**Catalog Number** 88-50440

**Pub. No.** MAN0016755  **Rev.** A.0 [10]

**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](https://thermofisher.com/support).

**Standard curve of IgG3 Mouse 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.

![Standard curve graph](image)

**Product information**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Contents</th>
<th>IgG3 Mouse Uncoated ELISA Kit with Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>REF</strong></td>
<td>Catalog number</td>
<td>88–50440</td>
</tr>
<tr>
<td>—</td>
<td>Sensitivity</td>
<td>1.875 ng/ml</td>
</tr>
<tr>
<td>—</td>
<td>Standard curve range</td>
<td>1.875–125.00 ng/ml</td>
</tr>
<tr>
<td>—</td>
<td>Temperature limitation</td>
<td>Store at 2–8°C</td>
</tr>
<tr>
<td><strong>LOT</strong></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 IgG3 Mouse 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 mouse IgG3 protein levels from samples including serum, plasma and supernatants from cell cultures.

**Components of 2-plate format (2x96 tests)**

- **Capture Antibody:** Pre-titrated, purified anti-mouse IgG3 monoclonal antibody
  
  1 vial (100 µL) Capture Antibody Concentrate (250x)

- **Detection Antibody:** Pre-titrated, HRP-conjugated anti-mouse IgG polyclonal antibody
  
  1 vial (50 µL) Detection Antibody Concentrate (250x)

- **Standard:** 2 vials mouse IgG3 isotype control (standard), lyophilized, 250 ng/ml upon reconstitution

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

- **Assay Buffer A:** 2 bottles (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-mouse IgG3 monoclonal antibody
  
  1 vial (500 µL) Capture Antibody Concentrate (250x)

- **Detection Antibody:** Pre-titrated, HRP-conjugated anti-mouse IgG polyclonal antibody
  
  1 vial (250 µL) Detection Antibody Concentrate (250x)

- **Standard:** 10 vials mouse IgG3 isotype control (standard), lyophilized, 250 ng/ml upon reconstitution

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

- **Assay Buffer A:** 2 bottles (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)

**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
Experimental procedure

**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:** If crystals form in the buffer concentrates, warm them gently until they completely dissolve.

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 mouse IgG3 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 insure complete and homogeneous solubilization (concentration of reconstituted standard = 250 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).

7. **Sample Preparation**
   - 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 A. For serum and plasma samples our recommended starting dilution is 10,000-fold in Assay Buffer A. This dilution was obtained by using samples from CD-1 mice.
   - Note that the dilution indicated above is only our recommendation.

8. **Blank**
   - Add 100 µL/well of Assay Buffer A (1x) to the blank wells.

9. **Sample Dilution**
   - Add 100 µL of Assay Buffer A (1x) to all standard wells.
   - Add 100 µL reconstituted standard in duplicate into well A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1=125 ng/ml and transfer 100 µL to wells B1 and B2, respectively. Take care not to scratch surface of the microwells. Continue this procedure five times.

10. **Sample Dilution**
    - For that add 100 µL of Assay Buffer A (1x) to the sample wells.

11. **Buffer A Concentrate (20x)**
    - Dilution of other biological samples needs to be determined empirically.

12. **Wash**
    - Add 50 µL/well of Assay Buffer A (1x) to make the standard curve.
    - Add 100 µL reconstituted standard in duplicate into well A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1=125 ng/ml) and transfer 100 µL to wells B1 and B2, respectively. Take care not to scratch surface of the microwells. Continue this procedure five times.

13. **Aspirate/wash**
    - Aspirate/wash as in step 3. Repeat for a total of two washes.

14. **Sample Preparation**
    - Perform 2-fold serial dilutions of the standards with Assay Buffer A (1x) to make the standard curve.
    - For that add 100 µL of Assay Buffer A (1x) to all standard wells. Add 100 µL reconstituted standard in duplicate into well A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1=125 ng/ml) and transfer 100 µL to wells B1 and B2, respectively. Take care not to scratch surface of the microwells. Continue this procedure five times.

15. **Aspirate/wash**
    - Aspirate/wash as in step 3. Repeat for a total of two washes.

16. **Buffer A (1x)**
    - For other biological samples needs to be determined empirically.

17. **Aspirate/wash**
    - Aspirate/wash as in step 3. Repeat for a total of two washes.

18. **Buffer A (1x) to the blank wells.

19. **Sample Dilation**
    - Add 100 µL/well of Assay Buffer A (1x) to all standard wells.

20. **Buffer A (1x) to the blank wells.

21. **Aspirate/wash**
    - Aspirate/wash as in step 3. Repeat for a total of two washes.

22. **Sample Dilation**
    - Add 100 µL/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.

23. **Read**
    - Add 100 µL of Stop Solution to each well.

24. **Read**
    - Read plate at 450 nm. If wavelength substraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

**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 serum and plasma samples have been diluted 1:20,000, the concentration read from the standard curve must be multiplied by the dilution factor (x20,000).

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).

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. Incubate at room temperature for 2 hours (or over night 4°C).

5. Prepare the Standard and Detection Antibody (see points 5 and 6 in Reagent preparation).

6. Aspirate/wash as in step 3. Repeat for a total of two washes.

7. Perform 2-fold serial dilutions of the standards with Assay Buffer A (1x) to make the standard curve.
## ELISA troubleshooting guide

<table>
<thead>
<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>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>Very weak signal</td>
<td>Improper and inefficient washing.</td>
<td>Make sure washing procedure is done correctly.</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>Variation of kit performance</td>
<td>Different buffers, plates. Handling can strongly affect kit performance.</td>
<td>Use eBioscience™ buffers, plates, and kit components available.</td>
</tr>
</tbody>
</table>

### Customer and technical support
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- Worldwide contact telephone numbers
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  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- 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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