IgG2 Human Uncoated ELISA Kit with Plates

Enzyme-linked immunosorbent assay for quantitative detection of human IgG2

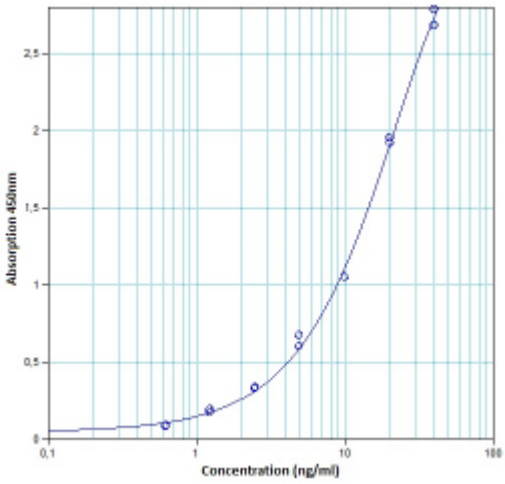
Catalog Number 88-50570

Pub. No. MAN0016749 **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**.

Standard curve of IgG2 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

Symbol	Contents	IgG2 Human Uncoated ELISA Kit with Plates
REF	Catalog number	88–50570
—	Sensitivity	0.63 ng/ml
—	Standard curve range	40–0.63 ng/ml
	Temperature limitation	Store at 2–8°C
LOT	Batch code	Refer to vial
	Use by	Refer to box label
	Caution	Contains preservatives

Description

This IgG2 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 IgG2 protein levels from samples including serum and plasma.

Components of 2-plate format (2x96 tests)

Capture Antibody: Pre-titrated, purified anti-human IgG2 monoclonal antibody

1 vial (100 µL) Capture Antibody Concentrate (250x)

Detection Antibody: Pre-titrated, HRP-conjugated anti-human IgG monoclonal antibody

1 vial (100 µL) Detection Antibody Concentrate (250x)

Standard: Recombinant human IgG2 for generating standard curve and calibrating samples

2 vials human IgG2 Standard (lyophilized): 80 ng/ml upon reconstitution

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

Assay Buffer A: 3 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

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 H_3PO_4 or 2 N H_2SO_4 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: 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 human IgG2 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 = 80 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 100,000-fold in Assay Buffer A.

   Note that the dilution indicated above is only our recommendation.

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 serum and plasma samples have been diluted 1:500,000, the concentration read from the standard curve must be multiplied by the dilution factor (x500,000).

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 (see point 5 of 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.

   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=40 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.
8. Add 100 µL/well of Assay Buffer A (1x) to the blank wells.
9. Add 80 µL/well of Assay Buffer A (1x) to the sample wells.
10. Add 20 µL/well of your prediluted serum or plasma samples to the appropriate wells, prediluting them at least 100,000-fold in Assay Buffer A (1x). Dilution of other biological samples needs to be determined empirically.
11. Cover or seal the plate and incubate at room temperature for 2 hours, if available on a microplate shaker set at 400 rpm.
12. Aspirate/wash as in step 3. Repeat for a total of four washes.
13. Prepare the Detection Antibody (see point 6 of Reagent Preparation).
14. Add 100 µL/well diluted Detection Antibody to all wells.
15. Cover or seal the plate and incubate at room temperature for 1 hour, if available on a microplate shaker set at 400 rpm.
16. Aspirate/wash as in step 3. Repeat for a total of four washes.
17. Add 100 µL/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
18. Add 100 µL of Stop Solution to each well.
19. Read plate at 450 nm. If wavelength substraction is available, substract the values of 570 nm from those of 450 nm and analyze data.

ELISA troubleshooting guide

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

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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

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