

PRODUCT INFORMATION & MANUAL

Human IgG4 Ready-SET-Go! ®

88-50590

Ready-SET-Go! Enzyme-linked Immunosorbent Assay for quantitative detection of human IgG4.
For research use only.



Human IgG4 Ready-SET-Go! ELISA

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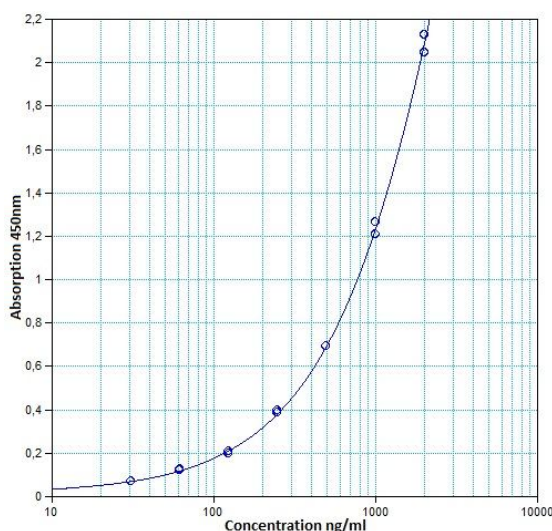
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Human IgG4 ELISA Ready-SET-Go!®

Catalog Number: 88-50590

RUO: For Research Use Only



Standard curve of Human IgG4 ELISA Ready-SET-Go!®

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

Contents: Human IgG4 ELISA Ready-SET Go!®

REF **Catalog Number:** 88-50590

Sensitivity: 31.3 ng/ml

Standard Curve Range: 2 000 – 31.3 ng/ml



Temperature Limitation: Store at 2-8°C



Batch Code: Refer to Vial



Use By: Refer to box label



Caution, contains preservatives

Description

This Human IgG4 Ready-SET-Go! ELISA Set 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 IgG4 protein levels from samples including serum and plasma.

Components of 2-plate format (2x96 tests)

Capture Antibody: Pre-titrated, purified anti-human IgG4 monoclonal antibody

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

Detection Antibody: Pre-titrated, HRP-conjugated anti-human IgG4 monoclonal antibody

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

Standard: Recombinant human IgG4 for generating standard curve and calibrating samples

2 vials human IgG4 Standard (lyophilized): 4 000 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

TDS Protocol

Research Use Only

Other Materials Needed

- Buffers:
 - Wash Buffer: 1x PBS, 0.05% Tween-20 or eBioscience Wash Buffer (20x) cat. BMS408.0500
 - Stop Solution: 1M H₃PO₄ or 2N H₂SO₄ or eBioscience Stop Solution cat. BMS409.0100

- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning Costar 9018)

NOTE: The use of ELISA plates which 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 this ELISA Ready-SET-Go! Set, please only use the components included in the set. Exchanging of components is not recommended as a change in performance may occur.

Stability

This ELISA set 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 Set Reagents

Store at 2-8°C.

TDS Protocol

Research Use Only

Reagent Preparation

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 IgG4 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 = 4 000 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).

TDS Protocol

Research Use Only

Experimental Procedure

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 **Blocking Buffer** (see point 2 of 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 **Standard** (see point 5 of Reagent Preparation)
6. Aspirate/wash as in step 3. Repeat for a total of 2 washes.
7. Perform 2-fold serial dilutions of the **standards** with **Assay Buffer A** to make the standard curve:
For that add 100 µl of Assay Buffer A to all standard wells. Add 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=2 000 ng/ml) and transfer 100 µl to wells B1 and B2, respectively. Take care not to scratch surface of the microwells. Continue this procedure 5 times.
8. Add 100 µl/well of **Assay Buffer A** to the **blank wells**.
9. Add 90 µl/well of **Assay Buffer A** to the **sample wells**.
10. Add 10 µl/well of your prediluted **samples** to the appropriate wells, prediluting them at least 100-fold in Assay Buffer A**
11. Cover or seal the plate and incubate at room temperature for 2 hours.

12. Prepare **Detection Antibody** (see point 6 of Reagent Preparation)
13. Aspirate/wash as in step 3. Repeat for a total of 4 washes.
14. Add 100 µl/well diluted Detection Antibody.
15. Cover or seal the plate and incubate at room temperature for 1 hour.
16. Aspirate/wash as in step 3. Repeat for a total of 4 washes.
17. Add 100 µl/well of **Substrate Solution** to each well. Incubate plate at room temperature for approximately 15 minutes.
18. Add 100 µl of Stop Solution to each well.
19. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

*** 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 1:1 000, the concentration read from the standard curve must be multiplied by the dilution factor (x1 000).**

TDS Protocol

Research Use Only

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive controls	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with manufacturer
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA, using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor.

Problem	Possibility	Solution
C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly.
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis.
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each steps incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the frost free freezer
	5. Wrong filter in ELISA reader was used	5. Use correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date

Problem	Possibility	Solution
Variation of kit performance	1. Different buffers, plates 2. Handling can strongly affect kit performance	1. Use eBioscience buffers, plates and kit components available.