PRODUCT INFORMATION & MANUAL

Rat IgG2c Ready-SET-Go! ®

88-50530

Ready-SET-Go! Enzyme-linked Immunosorbent Assay for quantitative detection of rat IgG2c. For research use only.

eBioscience®
An Affymetrix Company

Rat IgG2c Ready-SET-Go! ELISA

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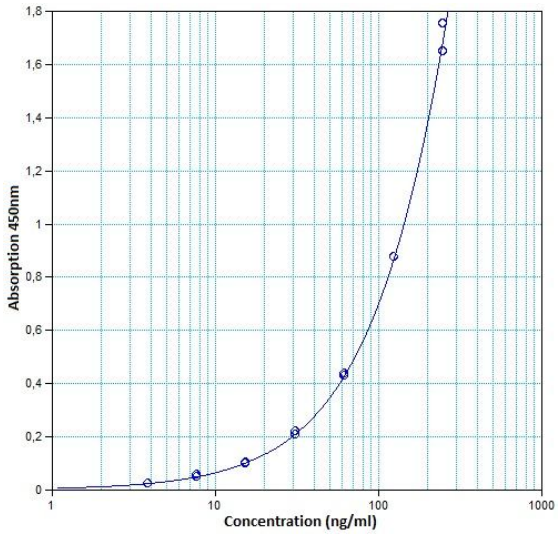
Rat IgG2c ELISA Ready-SET-Go!®

Catalog Number: 88-50530

RUO: For Research Use Only



Standard curve of Rat IgG2c 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: Rat IgG2c ELISA Ready-SET Go!®

REF **Catalog Number:** 88-50530

Sensitivity: 4 ng/ml

Standard Curve Range: 250 - 4 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 Rat IgG2c 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 rat IgG2c 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-rat IgG2c monoclonal antibody

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

Detection Antibody: Pre-titrated, HRP-conjugated anti-rat Ig(H+L) monoclonal antibody

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

Standard: Recombinant rat IgG2c for generating standard curve and calibrating samples

2 vials rat IgG2c Standard (lyophilized): 500 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-rat IgG2c monoclonal antibody

1 vial (500 µl) Capture Antibody Concentrate (250 x)

Detection Antibody: Pre-titrated, HRP-conjugated anti-rat Ig(H+L) monoclonal antibody

1 vial (500 µl) Detection Antibody Concentrate (250x)

Standard: Recombinant rat IgG2c for generating standard curve and calibrating samples

10 vials rat IgG2c Standard (lyophilized): 500 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)

10 96-well plates (included with product Cat.#'s ending in suffixes -76, -86)

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 H_3PO_4 or 2N H_2SO_4 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**

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 rat IgG2c 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 = 500 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=250 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 10 000-fold (1:100 > 1:100) 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 of diluted **Detection Antibody** to all wells.
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 substraction is available, substract the values of 570 nm from those of 450 nm and analyze data.

*** NOTE: If instructions of this protocol have been followed samples have been diluted 1:100 000, the concentration read from the standard curve must be multiplied by the dilution factor (x100 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. Contamined 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, avoide 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 perfomance	1. Use eBioscience buffers, plates and kit components available.