PRODUCT INFORMATION & MANUAL

Rat IgG2a Ready-SET-Go! ®

88-50510

Ready-SET-Go! Enzyme-linked Immunosorbent Assay for quantitative detection of rat IgG2a. For research use only.

eBioscience®
An Affymetrix Company

Rat IgG2a Ready-SET-Go! ELISA

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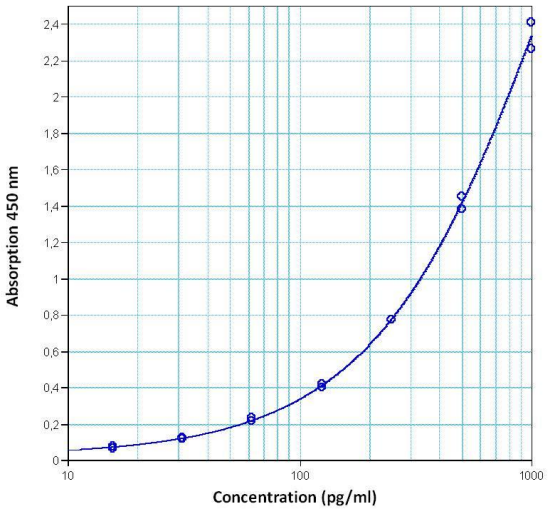

Standard curve of Rat IgG2a ELISA Ready-SET-Go!®

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Rat IgG2a ELISA Ready-SET Go!®

REF 88-50510

0.39 ng/ml

25 – 0.39 ng/ml

Store at 2-8°C

LOT Refer to Vial

Refer to box label

This Rat IgG2a 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 IgG2a protein levels from samples including serum, plasma and supernatants from cell cultures.

Pre-titrated, purified anti-rat IgG2a monoclonal antibody

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

Pre-titrated, HRP-conjugated anti-rat IgG(H+L) monoclonal antibody

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

Recombinant rat IgG2a for generating standard curve and calibrating samples

2 vials rat IgG2a Standard (lyophilized): 50 ng/ml upon reconstitution

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

2 bottles (10 ml) Assay Buffer A Concentrate 20x (PBS with 1% Tween 20 and 10% BSA)

Tetramethylbenzidine (TMB) Substrate Solution

1 bottle (25 ml)

2 96-well plates

Pre-titrated, purified anti-rat IgG2a monoclonal antibody

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

Pre-titrated, HRP-conjugated anti-rat IgG(H+L) monoclonal antibody

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

Recombinant rat IgG2a for generating standard curve and calibrating samples

10 vials rat IgG2a Standard (lyophilized): 50 ng/ml upon reconstitution

1 vial (12 ml) Phosphate Buffered Saline Concentrate (PBS) 10x

2 bottles (50 ml) Assay Buffer A Concentrate 20x (PBS with 1% Tween 20 and 10% BSA)

Tetramethylbenzidine (TMB) Substrate Solution

1 bottle (120 ml)

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

- 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)

- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

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.

Store at 2-8°C.

Make a 1:10 dilution of PBS (10x) in deionized water.

Make a 1:10 dilution of Assay Buffer A Concentrate (20x) in deionized water.

Make a 1:20 dilution of Assay Buffer A Concentrate (20x) in deionized water.

Dilute capture antibody (250x) 1:250 in Coating Buffer (1x).

Reconstitute rat IgG2a 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 = 50 ng/ml).

Mix well prior to making dilutions. The standard has to be used immediately after reconstitution and cannot be stored.

Dilute detection antibody (250x) 1:250 in Assay Buffer A (1x).

1. Coat Corning Costar 9018 ELISA plate with 100 µl/well of capture antibody in (dilute as noted in point 1 of Reagent Preparation). Seal the plate and incubate overnight at 4°C.
2. Prepare (see point 2 of Reagent Preparation)
3. Aspirate wells and wash twice with 400 µl/well . 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 (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 with 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= 25 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 to the .
9. Add 90 µl/well of to the .
10. Add 10 µl/well of your prediluted to the appropriate wells, prediluting them at least 100,000-fold (1:100 > 1:100 > 1:10) 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. . 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 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.

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.