PRODUCT INFORMATION & MANUAL

Rat Ig Isotyping
Ready-SET-Go! ®

88-50640

Ready-SET-Go! Enzyme-linked Immunosorbent Assay for the identification of rat Immunoglobulin isotypes. For research use only.

eBioscience®
An Affymetrix Company

Rat Ig Isotyping Ready-SET-Go! ELISA

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:
877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:
888.999.1371
858.642.2058
info@eBioscience.com

Fax:
858.642.2046

Europe/International*

Technical Support:
+43 1 796 40 40 120
tech@eBioscience.com

Customer Service:
+43 1 796 40 40 304
info@eBioscience.com

Fax:
+43 1 796 40 40 400

Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

* Customers in countries where direct sales are not available may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

Rat Ig Isotyping ELISA Ready-SET-Go!®
Catalog Number: 88-50640
RUO: For Research Use Only

Figure 1: Suggested Plate Layout for Rat Isotyping Kit

P = Positive Control   N = Negative Control   S1-S10 = Samples

Product Information

Contents: Rat Ig Isotyping ELISA Ready-SET Go!®

Analytes: IgA, IgG1, IgG2a, IgG2b, IgG2c, IgM, kappa-chain, lambda-chain

REF **Catalog Number:** 88-50640

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 Ig Isotyping Ready-SET-Go! ELISA Set contains the necessary reagents, controls, buffers and diluents for performing qualititative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for measurement of rat Immunoglobulins from supernatants from cell cultures.

Components of 10-plate format (10x96 tests)

Capture Antibody: Pre-titrated, purified anti-rat Ig monoclonal antibodies:

Quantity	Description	Concentration	Volume
1	mouse anti-rat kappa light chain	250x	100µl
1	mouse anti-rat lambda light chain	250x	100µl
1	mouse anti-rat IgG1	250x	100µl
1	mouse anti-rat IgG2a	250x	100µl
1	mouse anti-rat IgG2b	250x	100µl
1	mouse anti-rat IgG2c	250x	100µl
1	mouse anti-rat IgA	250x	100µl
1	mouse anti-rat IgM	250x	100µl

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

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

Control: Rat Ig isotype control mixture for generating positive controls

10 vials rat Ig Positive Controls (lyophilized)

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

Assay Buffer A: 1 bottle (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 (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. Positive Controls

Reconstitute Controls by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Allow the controls to reconstitute for 10-30 minutes. Swirl or mix gently to insure complete and homogeneous solubilization

Controls have 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). According to the suggested template (Fig. 1) coat each row with an other isotype-specific coating antibody starting with mouse anti-rat IgG1 in row A . 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 **Positive Control** (see point 5 of Reagent Preparation)

6. Aspirate/wash as in step 3. Repeat for a total of 2 washes.

7. Add 50 µl/well of **Assay Buffer A (1x)** to all wells.

8. Add 50 µl/well of your **samples** to the sample wells: According to the suggested template add 50 µl of sample 1 to each well of plate column 1, 50µl of sample 2 to each well of plate column 2. Repeat 8 more times for plate columns 3-10.

9. Add 50 µl **Positive Control** to each well of column 11.

10. Add 50 µl Negative Control (cell culture medium) to each well of plate column 12.

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 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.

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.