Technical Data Sheet

Purified Rat Anti-Mouse IgE

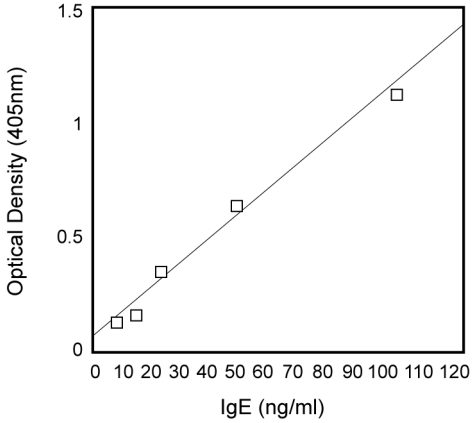
Product Information

Material Number:	**553413**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	R35-72
Immunogen:	Mouse IgE (pooled)
Isotype:	Rat (LOU) IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The rat anti-mouse IgE antibody (clone R35-72) reacts specifically with mouse IgE of the Igh-C [a] and Igh-C [b] haplotypes. It has been reported not to react with other Ig isotypes. Detection with the rat anti-mouse IgE antibody (clone R35-72) of surface immunoglobulin on IgE-secreting hybridoma cells has also been reported.

This antibody is routinely tested by ELISA analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



IgE standard curve obtained using purified rat anti-mouse IgE antibody (clone R35-72) at 2 µg/ml for capture and biotin rat anti-mouse IgE antibody (clone R35-118) at 2 µg/ml for detection of the mouse IgE standard (Cat. No. 557079).

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

ELISA Capture	Routinely Tested

Recommended Assay Procedure:

Sandwich ELISA: Purified rat anti-mouse IgE (clone R35-72) may be used at ~2 µg/ml as the capture antibody coupled with biotin rat anti-mouse IgE (clone R35-118) (Cat. No. 553419) as the detection antibody. Researchers are strongly advised to titrate each reagent over a range of concentrations (e.g 1-8 µg/ml) for optimal results. Purified mouse IgE (Cat. No. 557079, 553481, or 557080) may be used as the ELISA standard. Alternatively, the BD OptEIA™ Mouse IgE ELISA Set (Cat. No. 555248) is offered as a convenient sandwich ELISA product that is easy-to-use and may be used for the quantitation of soluble mouse IgE.

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Mouse IgE ELISA Protocol

I. Coat with capture antibody:

1. Dilute the purified rat anti-mouse IgE capture antibody (clone R35-72) (Cat. No. 553413) to ~ 2 µg/ml in coating buffer. Add 100 µl per well to an enhanced protein-binding ELISA-grade plate (e.g., BD Falcon™ Cat. No. 353279). Investigators are encouraged to to determine the optimal antibody concentration for their use. Titrations between 1-8 µg/ml are suggested.
2. Shake plate to ensure all wells are covered by the capture antibody solution.
3. Cover the plate and incubate for 1 hour at 37°C or overnight at 4°C.
4. Wash the plate 3X with PBS/Tween. For each wash, wells are filled with 200 µl PBS/Tween and allowed to stand at least 1 minute prior to aspirating or dumping. As a final step, tap plate on paper towels to remove excess buffer.

II. Blocking:

1. Block the plate with 200 µl blocking buffer per well.
2. Cover the plate and incubate at room temperature for 30 minutes.
3. Wash the plate 3X with PBS/Tween, as in described section I, step 4.

III. Apply standards and samples:

1. Leave column 1 of the plate as blank wells (i.e., no antigen added at 100 µl per well consisting of blocking buffer only). Use columns 2 and 3 for duplicates of the standard at 100 µl per well. Dilute the purified mouse IgE standard (Cat. No. 557079, 553481 or 557080) in blocking buffer. Dilutions should range in a series of 8 two-fold dilutions, in blocking buffer, starting at 0.5 µg/ml. Use the remaining columns to add samples of interest at various dilutions in blocking buffer at 100 µl per well.
2. Cover the plate and incubate for at least 1 hour at room temperature or overnight at 4°C.
3. Wash the plate 3X with PBS/Tween, as in section I, step 4.

IV. Incubation with detection antibody:

1. Dilute the biotinylated rat anti-mouse IgE antibody (clone R35-118) (Cat. No. 553419) to ~ 2 µg/ml in blocking buffer. Add 100 µl per well. Investigators are encouraged to to determine the optimal antibody concentration for their use. Titrations between 1-8 µg/ml are suggested.
2. Cover the plate and incubate at room temperature for 1 hour.
3. Wash the plate 6X with PBS/Tween, as in section I, step 4.

V. Add avidin- or streptavidin-horseradish peroxidase (Av-HRP or SAv-HRP):

1. Dilute Av-HRP (Cat. No. 554058) or SAv-HRP (Cat. No. 554066) as recommended for the product (e.g 1:1000) in blocking buffer. Add 100 µl per well.
2. Cover the plate and incubate at room temperature for 30 minutes.
3. Wash the plate 6X with PBS/Tween, as in section I, step 4.

VI. Add substrate and develop:

1. Thaw substrate (ABTS) buffer within 20 minutes of use. Add 11 µl of 30% H2O2 (Sigma-Aldrich, Cat. No. H1009) to 11 ml substrate buffer and vortex. Immediately add 100 µl per well and allow to develop at room temperature for 20-30 minutes. Color reaction can be stopped by adding 50 µl per well of SDS/DMF Solution (optional).
2. Read the plate at 405 nm.

*SOLUTIONS

Coating Buffer
PBS, pH 7.2 - 7.4

PBS Solution

NaCl	80.0 g
Na_2HPO_4	11.6 g
KH_2PO_4	2.0 g
KCl	2.0 g

ddH_2O to 10 liter
Adjust pH to 7.2 - 7.4

PBS/Tween
PBS
Tween-20 0.05%

Blocking Buffer
PBS
Fetal calf serum 10%
or BSA 1%

Substrate Buffer

ABTS (3-ethylbenzthiazoline-6-sulfonic acid, Sigma Cat. no. A-1888)	150 mg
0.1 M citric acid (eg, Fisher anhydrous, Cat. no. A-940)	500 ml

Adjust pH to 4.35 with NaOH pellets
Aliquot at 11 ml per vial and store at -20°C

SDS/DMF Solution
40% SDS (80 g SDS in 200 ml dd H_2O)
Add 200 ml DMF (N.N-dimethyl formamide)

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Suggested Companion Products

Catalog Number	Name	Size	Clone
553419	Biotin Rat Anti-Mouse IgE	0.5 mg	R35-118
554066	Streptavidin HRP	1.0 ml	(none)
557079	Purified Mouse IgE κ Isotype Control	0.5 mg	C38-2

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE™ (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

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