Technical Data Sheet

Purified Mouse IgE, κ Isotype Standard

Product Information

Material Number:	**553481**
Alternate Name:	5-dimethylaminonapthalene-1-sulfonyl (dansyl)
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	27-74
Isotype:	Mouse (C.SW) IgE, κ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 27-74 antibody is specific for the hapten 5-dimethylaminonapthalene-1-sulfonyl (dansyl).

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 Standard	Routinely Tested

Recommended Assay Procedure:
Clone 27-74 is useful as a standard in ELISA. Please refer to the Mouse IgE ELISA Protocol below.

MOUSE IgE ELISA PROTOCOL

Coat with Capture Antibody:
1. Dilute the purified anti-mouse IgE capture mAb (Cat. No. 553413, clone R35-72) to 2 µg/ml in coating buffer.* Add 100 µl per well to an enhanced protein-binding ELISA plate (e.g., BD Falcon™ ELISA Plates, BD Labware Cat. No. 353279).
2. Shake plate to ensure all wells are covered by 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.

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 Section I, Step 4, of this protocol.

Apply Standards and Samples:
1. Leave column 1 as blank wells (i.e., no antigen added, 100 µl per well blocking buffer only). Use columns 2 and 3 for duplicates of the standard, 100 µl per well: dilute purified mouse IgE standard (Cat. No. 557079, clone C38-2; or Cat. No. 553481, clone 27-74) or mouse IgE standard (Cat. No. 557080, clone C48-2) in a series of 8 two-fold dilutions, in blocking buffer, starting at 0.5 µg/ml. Use the remaining columns to add samples at various dilutions in blocking buffer, 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, of this protocol.

Incubation with Detection Antibody:
1. Dilute biotinylated anti-mouse IgE (Cat. No. 553419, clone R35-118) to 2 µg/ml in blocking buffer. Add 100 µl per well.
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, of this protocol.

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Add Avidin- or Streptavidin-Horseradish Peroxidase (Av-HRP or SAv-HRP):

1. Dilute Av-HRP (Cat. No. 554058) or SAv-HRP (Cat. No. 554066) 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, of this protocol.

Add Substrate and Develop:

1. Thaw substrate (ABTS) buffer* within 20 minutes of use. Add 11 µl of 30% H 2O2 (Sigma, 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 50µl per well of SDS/DMF Solution* (optional).
2. Read the plate at 405 nm.

***SOLUTIONS**

Coating Buffer
PBS, pH 7.2 - 7.4

PBS/Tween
PBS
Tween-20 0.05%

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

PBS Solution
NaCl 80.0 g
Na2HPO4 11.6 g
KH2PO4 2.0 g
KCl 2.0 g
ddH2O to 10 liter
Adjust pH to 7.2 - 7.4

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

SDS/DMF Solution
40% SDS (80 g SDS in 200 ml dd H2O)
Add 200 ml DMF (N.N-dimethyl formamide)

NOTES

a. In most cases, coating the plate with primary mAb at 2 µg/ml, 100 µl per well and detecting with the biotinylated secondary mAb at 2 µg/ml, 100 µl per well yields a very satisfactory signal. However, for optimal signal, researchers should titrate each mAb over a range of concentrations (eg, 1 - 8 µg/ml).
b. Recommended incubation conditions for optimal sensitivity.
c. Streptavidin/Avidin-HRP conjugate from another supplier may be substituted and diluted according to the manufacturer's recommendation.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553413	Purified Rat Anti-Mouse IgE	0.5 mg	R35-72
553419	Biotin Rat Anti-Mouse IgE	0.5 mg	R35-118
554066	Streptavidin HRP	1.0 ml	(none)

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.

References

Dangl JL, Parks DR, Oi VT, Herzenberg LA. Rapid isolation of cloned isotype switch variants using fluorescence activated cell sorting. *Cytometry*. 1982; 2(6):395-401.(Immunogen)

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