Technical Data Sheet

Purified Rat Anti-Mouse C5a

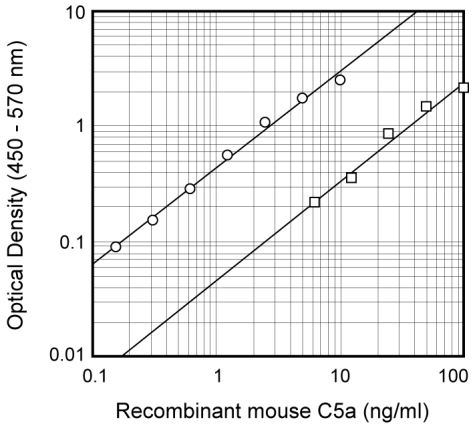
Product Information

Material Number:	**558027**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	I52-1486
Immunogen:	Recombinant mouse C5a
Isotype:	Rat IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The purified I52-1486 antibody reacts with the mouse C5a protein. Anaphylatoxin C5a is a bioactive cleavage product released from plasma component C5 during complement activation and is involved in mediation of a variety of cellular immune responses, as well as being potent pro-inflammatory agents. The release of this cleavage product is a reliable indicator of in vivo or in vitro complement activation. Clone I52-1486 hybridoma was generated by the fusion of spleen cells from rats immunized with recombinant mouse C5a protein. The I52-1486 antibody is specific for a neoepitope exposed in mouse C5a/C5adesArg and does not cross-react with mouse C5. This capacity makes antibody I52-1486 a preferential capture antibody for direct detection of mouse C5a from plasma or serum samples.

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



ELISA curves from a sandwich ELISA that measures mouse C5a protein levels. *The curves were generated by a sandwich ELISA using the purified I52-1486 antibody as capture antibody, doubling dilutions of recombinant mouse C5a protein as standard and biotinylated I52-278 antibody as detection antibody. Avidin-HRP and the TMB substrate were used to develop the detection stage and mean OD was measured at 450-570 nm. The standard curve is displayed as the concentration of recombinant mouse C5a in ng/ml versus the microwell absorbance (in circles). Doubling dilutions of normal mouse serum, starting at 1:2 dilution, are also shown (squares).*

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

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Recommended Assay Procedure:
The purified I52-1486 antibody is useful as a capture antibody for a sandwich ELISA that measures mouse C5a protein levels in plasma or serum samples. Purified I52-1486 as capture antibody can be paired with the biotinylated I52-278 anti-mouse C5a (Cat. No. 558028) as the detection antibody, using recombinant mouse C5a protein (Cat. No. 622597) as the standard. Addition of FUT-175 (Futhan, Cat. No. 552035) to plasma samples at the time of sample collection provides additional protection from ex-vivo activation, and therefore ensures more accurate measurements that reflect the circulating levels of complement activation products. The purified I52-1486 antibody should be titrated between 1-4 µg/ml, diluted in carbonate buffer pH=9.5, to determine the optimal ELISA plate-coating concentration. For blocking buffer, PBS containing 10% FCS is recommended (Cat. No. 555213). To obtain linear standard curves, doubling dilutions of recombinant mouse C5a protein ranging from 156 pg to 10 ng/ml are recommended for inclusion in each ELISA plate. For specific methodology and buffer recipes, see Chapter 7: ELISA for specifically measuring the levels of cytokines, chemokines, and inflammatory mediators and their receptors. 2003. Techniques for Immune Function Analysis Application Handbook 1st Edition. BD Biosciences. This ELISA antibody pair shows no cross-reactivity with the following: recombinant mouse C3a, native human, and rat C5a.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558028	Biotin Rat Anti-Mouse C5a	0.1 mg	I52-278
622597	Purified Recombinant Mouse C5a	10 tests	(none)
550534	Reagent Set B	20 plates	(none)
555213	Assay Diluent	500 ml	(none)
555214	TMB Substrate Reagent Set	each	(none)
552035	FUT-175 (Futhan)	5.0 mg	(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.

References

Ember JA., Jagels MA., Hugli TE. Characterization of complement anaphylatoxins and their biological responses. In: J.E. Volanakis and M.M. Frank, ed. *The human complement system in health and disease.* Marcel Dekker, Inc; 1998:241-284.(Biology)

Hugli TE. Structure and function of the anaphylatoxins. *Springer Semin Immunopathol.* 1984; 7:193-219.(Biology)

Volanakis, J.E. Overview of the complement system. In.: The human complement system in health and disease. In: J.E. Volanakis and M.M. Frank, ed. Marcel Dekker, Inc.; 1998:9-32.(Biology)