

Technical Data Sheet

Biotin Rat Anti-Mouse C5a

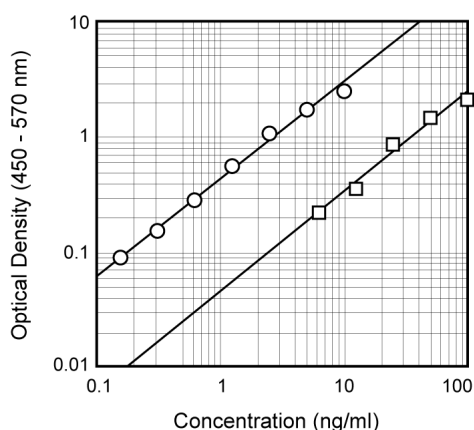
Product Information

Material Number:	558028
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	I52-278
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The I52-278 monoclonal antibody reacts with the mouse C5a protein. Clone I52-278 hybridoma was generated by the fusion of spleen cells from rats immunized with recombinant 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.

This antibody is routinely tested by ELISA detection. 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). 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.

The antibody was conjugated with biotin under optimum conditions, and unreacted biotin was removed.

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

ELISA Detection	Routinely Tested
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Recommended Assay Procedure:

The biotinylated I52-278 antibody is useful as a detector antibody for a sandwich ELISA that measures mouse C5a protein levels. Purified I52-1486 as capture antibody (Cat. No. 558027) can be paired with the biotinylated I52-278 anti-mouse C5a as the detection antibody, using purified recombinant mouse C5a protein (Cat. No. 622597) as standard. The biotinylated I52-278 antibody should be titrated between 0.25-2 μ g/ml to determine its optimal ELISA detection concentration. To obtain linear standard curves, doubling dilutions of purified mouse C5a protein ranging from 156 pg to 10 ng/ml are recommended for inclusion in each ELISA plate. PBS with 10%FCS is recommended as the blocking buffer. Addition of FUT-175 (Futhan), Cat. No. 552035, to plasma samples at the time of sample collection provides additional protection from ex-vivo

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activation, and therefore ensures more accurate measurements that reflect the circulating levels of complement activation products. For specific methodology and ELISA buffer recipes, see Immune Function Handbook which is posted on the website at www.bdbiosciences.com. This ELISA antibody pair shows no cross-reactivity with the following: recombinant mouse C3a, native human, and rat C5a.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
558027	Purified Rat Anti-Mouse C5a	0.1 mg	152-1486
622597	Purified Recombinant Mouse C5a	10 tests	(none)
550534	Reagent Set B	20 plates	(none)
552035	FUT-175 (Futhan)	5.0 mg	(none)
555213	Assay Diluent	500 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.

References

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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)