

Datasheet for ABIN2473066

**anti-Complement Factor Bb antibody****1** Validation**1** Publication[Go to Product page](#)

## Overview

Quantity:	0.1 mg
Target:	Complement Factor Bb
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Complement Factor Bb antibody is un-conjugated
Application:	Flow Cytometry (FACS), ELISA, Western Blotting (WB)

## Product Details

Clone:	032B-22-1-X
Isotype:	IgG2a
Specificity:	Specificity property: neoantigen The monoclonal antibody, clone 032B-22.1.X, binds to a neoantigen expressed on the Bb fragment of human Factor B. This antigen is not expressed on native Factor B and, accordingly, the antibody does not bind to native Factor B found in human plasma.
Characteristics:	Purified IgG
Purification:	Purified

## Target Details

Target: Complement Factor Bb

Abstract: [Complement Factor Bb Products](#)

## Target Details

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Gene ID: 629

UniProt: [P00751](#)

## Application Details

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Application Notes: Optimal working dilution should be determined by the investigator.

Comment: If the Factor B is denatured in any way, the protein can express neoantigen(s) that are not present in functionally active, native Factor B. For example, if the Factor B is bound to a well in a microtiter plate, a portion of it will be conformationally distorted and express the Bb neoantigen. Purification of Factor B can also result in denaturation of a portion of the Factor B and this denatured protein can also express the Bb-specific neoantigen. Finally, lack of careful handling of serum and plasma can result in complement activation and generation of small amounts of Bb and Ba fragments; in this case low levels of Bb will be detectable with this antibody and with the MicroVue assay for Bb.

Restrictions: For Research Use only

## Handling

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Format: Liquid

Concentration: 1.0 mg/mL

## Publications

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Product cited in: Xu, Narayana, Volanakis: "Structural biology of the alternative pathway convertase." in: **Immunological reviews**, Vol. 180, pp. 123-35, (2001) ([PubMed](#)).



**Successfully validated (Western Blotting (WB))**

by [Complement Physiopathology, Departamento de Microbiología I, Universidad Complutense de Madrid](#)

Report Number: 103567

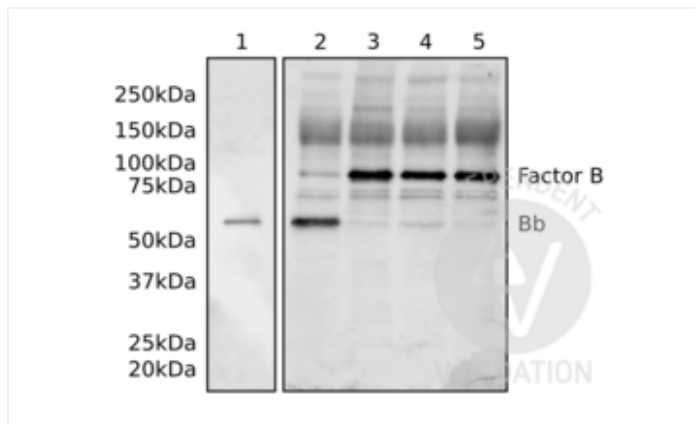
Date: Feb 22 2019

Target:	Bb
Lot Number:	146096
Method validated:	Western Blotting (WB)
Positive Control:	Purified Bb fragment and zymosan activated serum
Negative Control:	Non-activated sera
Notes:	ABIN2473066 recognizes both Factor B and Bb fragment in plasma samples. It does not specifically recognize a neo-epitope in Bb fragment.
Primary Antibody:	ABIN2473066
Secondary Antibody:	goat anti-mouse Immunoglobulins/HRP (Dako, P0447, lot 20058518)
Protocol:	<ul style="list-style-type: none"><li>• Purify Bb fragment from plasma samples.</li><li>• Separate 10ng purified Bb fragment and 0.5µl of zymosan activated serum and 0,5 µl of control serums on a 10% SDS-PAGE electrophoresis gel for 1h 120 V.</li><li>• Activate PVDF membrane with methanol for 15sec and rinse with transfer buffer for 5min. Transfer proteins from the gel to the PVDF membrane using a Mini Trans-Blot cell Tetra Blotting Module (Bio-Rad, 1660827EDU) for 1h at 100V.</li><li>• Stain membrane using Ponceau S to verify protein transfer.</li><li>• Block the membrane with blocking buffer (1x PBS, 3% BSA, 0.1% Tween) for 30min at RT.</li><li>• Incubate membrane with primary mouse anti-complement factor Bb antibody (antibodies-online, ABIN5692886, lot 146096) diluted 1:1000 in working buffer (1x PBS 1% BSA, 0.1% Tween) ON at 4°C.</li><li>• Wash membrane 3x for 5min with PBST (1x PBS, 0.1% Tween).</li><li>• Incubate membrane with secondary goat anti-mouse Immunoglobulins/HRP (Dako, P0447, lot 20058518) diluted 1:1000 in working buffer for 30min at RT.</li><li>• Wash membrane 3x for 5min with PBST.</li><li>• Reveal protein bands using Clarity™ Western ECL substrate (Bio-Rad, 170-5061, lot 102031078) on a ChemiDoc MP Imaging System (Bio-Rad, 17001402).</li></ul>
Experimental Notes:	<ul style="list-style-type: none"><li>• In denatured samples the complement Factor Bb antibody directed against a neo-epitope in Bb fragment ABIN2473066 reveals two proteins of the expected molecular weight of Factor B (90kDa) and Bb fragment (60kDa). The antibody does not specifically recognize a neo-</li></ul>

epitope in Bb fragment and is thus not suitable for selectively detecting the Bb fragment subsequently to denaturation.

- As an additional control we performed a Western Blot using in-house antibodies which detect Factor B and fragments Bb and Ba (not shown).

Image for Validation report #103567



**Validation image no. 1 for anti-Complement Factor Bb antibody (ABIN2473066)**

Western blot analysis of purified Bb fragment (1), zymosan activated serum (2), and non-activated sera (3, 4, 5) using ABIN5692886.