



IgMBRAZOR™

Digestion of IgM

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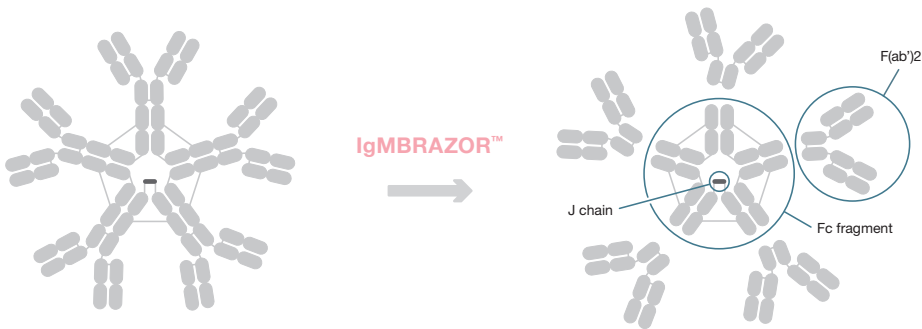
Digestion of IgM



IgMBRAZOR is a unique IgM-specific protease that digests human IgM at one specific site below the C_H2 domain in the heavy chain, generating homogeneous F(ab')₂ and Fc fragments. The enzyme enables middle-level analysis of the complex and high molecular weight human IgM, which facilitates IgM characterization during, for example, the development of vaccines, therapeutics, and diagnostics.

-  Human IgM
-  VPDQDT / AIRVFA (in the heavy chain)
-  30 min reaction
-  No reducing agents or co-factors needed

Digestion Workflow



IgMBRAZOR is a unique IgM-specific enzyme that digests human IgM at one specific site. The digestion is complete within 30 minutes, and due to the specificity of the enzyme, there is no risk of over-

digestion if the incubation time is prolonged. The enzyme is active at pH 5.5 to 9.0 and does not require reducing conditions or co-factors for activity. The optimal activity is at 37°C, but the

digestion can also be performed at room temperature using an increased incubation time.

Product Formats

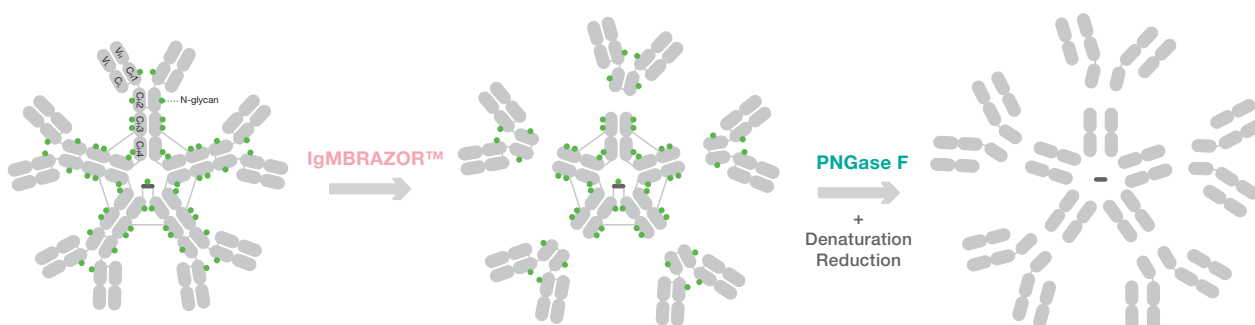


IgMBRAZOR™ Lyophilized
Lyophilized enzyme for digestion of IgM

IgMBRAZOR™ Lyophilized

PRODUCT	DESCRIPTION	ID
IgMBRAZOR Lyophilized 1000 units	Process 1 mg human IgM	M0-IM1-010

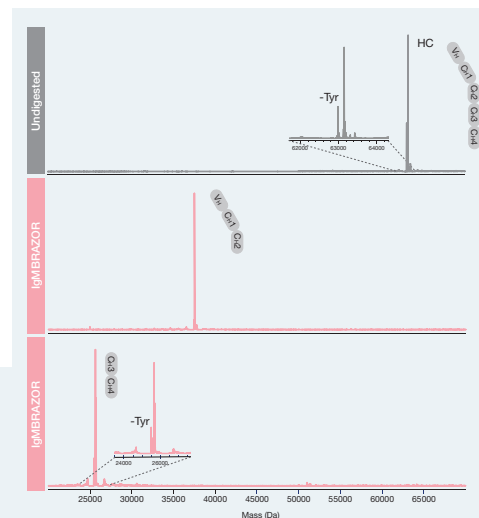
Site-specific Digestion of IgM



To demonstrate the site-specificity of IgMBRAZOR, human myeloma IgM was here digested with IgMBRAZOR Lyophilized for 30 minutes at 37°C and compared to undigested IgM. To facilitate data interpretation, the N-glycans were removed using PNGase F under denaturing and reducing conditions. The homogenous fragments observed in the mass spectra after IgMBRAZOR digestion and the masses of the detected fragments were

consistent with digestion at one single site (...VPDQDT/AIRVFA...). The digestion using IgMBRAZOR is fast, easy, and carried out under physiological conditions. Middle-level analysis of IgM allows for the generation of high resolution mass data with minimal sample preparation and data analysis, which can be efficiently used for characterization, quality control, stability testing and production monitoring of therapeutic antibodies.

Site-specific digestion of IgM. IgM was first digested with IgMBRAZOR Lyophilized for 30 minutes at 37°C, then the Fc N-glycans were removed using PNGase F Lyophilized under reducing and denaturing conditions to facilitate data interpretation. Deconvoluted mass spectra of the intact deglycosylated heavy chain and the V_H - C_H1 - C_H2 and C_H3 - C_H4 fragments generated after IgMBRAZOR and PNGase F digestion are shown in the figure to the right. The inserts (top and bottom spectra) display the two peaks with and without the loss of the C-terminal tyrosine. The analysis was performed by reversed-phase LC-MS using a Waters™ BioAccord™ LC-MS system.



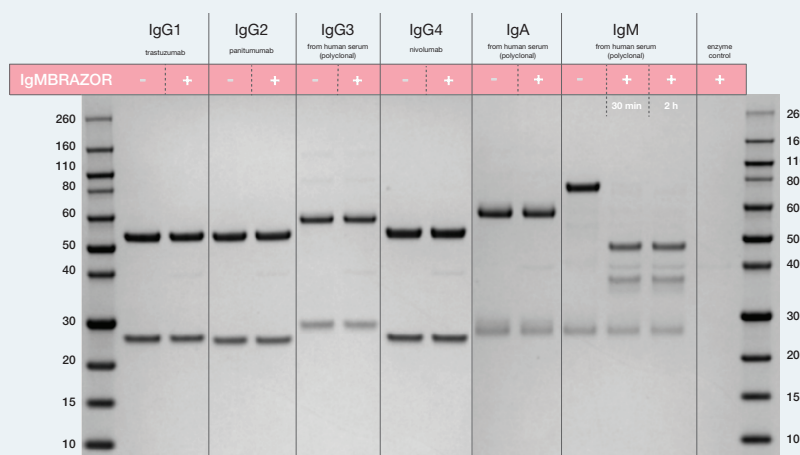
Isotype-specific Digestion using IgMBRAZOR™



IgMBRAZOR is isotype-specific towards human IgM antibodies. Here, all four subclasses of human IgG along with IgA and IgM were incubated with IgMBRAZOR Lyophilized. No digestion was observed

for any of the IgG subclasses or IgA, while IgM was successfully digested into F(ab')₂ and Fc fragments in 30 minutes. This shows the high specificity of IgMBRAZOR and demonstrates the

possibility of using the enzyme in complex samples such as serum, plasma and blood samples without the risk of off-target digestion.



Isotype specificity of IgMBRAZOR. Human IgG1 (trastuzumab), IgG2 (panitumumab), IgG3 (from human serum; polyclonal), IgG4 (nivolumab) and IgA (from human serum; polyclonal) were incubated with IgMBRAZOR Lyophilized for 2 h at 37°C. IgM (from human serum; polyclonal) was incubated with IgMBRAZOR Lyophilized for 30 minutes or 2 h at 37°C. All samples were analyzed reduced with SDS-PAGE.

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