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Datasheet for 009-0607

Human IgM (myeloma) Biotin

Overview

Description:	Human IgM (myeloma) Whole Molecule Biotin Conjugated - 009-0607
Item No.:	009-0607
Size:	1 mg
Applications:	Microarray, Other
Origin:	Human

Product Details

Background:	Immunoglobulin M is the largest antibody isotype and the first to be secreted against an initial exposure to antigen. IgM is predominantly produced in the spleen. Formed from covalently linking 5 immunoglobulins together, the approximate molecular weight of IgM is 900kDa and possesses 10 binding sites (though due to the size of most antigens, not all sites are capable of binding at once). Due to this large size, IgM is typically isolated to the serum.
Synonyms:	Human IgM whole molecule Biotin conjugated, human myeloma IgM whole molecule biotin conjugation
Species of Origin:	Human
Conjugate:	Biotin
Format:	IgM
Type:	Native Protein

Target Details

Purity/Specificity:	This product was prepared from normal serum by a multi-step process which includes delipidation, selective precipitation, ion exchange chromatography followed by tandem molecular sieve chromatography and extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-biotin, anti-Human IgM and anti-Human Serum. No reaction was observed against anti-Human IgG F(c) or anti-Pepsin.
Relevant Links:	• 009-0607 SDS

Application Details

Suggested Applications:	Microarray, Other (Based on references)
Application Note:	Human IgM (myeloma) Biotin conjugated whole molecule can be used as a control or standard in indirect trapping ELISA for quantitation of antigen in serum using a standard curve, for immunoprecipitation and for western blotting.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.

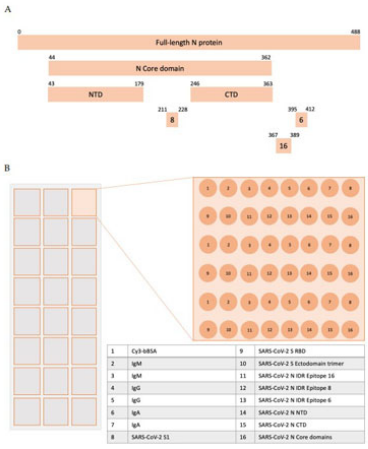
Formulation

Physical State:	Lyophilized
Concentration:	1.000 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	1.0 mL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

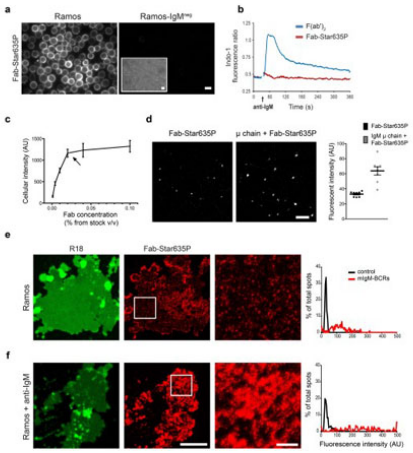
Images



1	Cy3-bBSA	9	SARS-CoV-2 S RBD
2	IgM	10	SARS-CoV-2 S Ectodomain trimer
3	IgM	11	SARS-CoV-2 N IDR Epitope 16
4	IgG	12	SARS-CoV-2 N IDR Epitope 8
5	IgG	13	SARS-CoV-2 N IDR Epitope 6
6	IgA	14	SARS-CoV-2 N NTD
7	IgA	15	SARS-CoV-2 N CTD
8	SARS-CoV-2 S1	16	SARS-CoV-2 N Core domains

Figure

SARS-CoV-2 N protein amino acid coverage on SARS-CoV-2 microarray. (A) Final N protein constructs fabricated on the SARS-CoV-2 microarray, including the full-length protein, core domain (amino acids 44-362), N-terminal domain (NTD) (amino acids 43-179), C-terminal domain (CTD) (amino acids 246-363), peptide 6 (amino acids 395-412), peptide 8 (amino acids 211-228) and peptide 16 (amino acids 367-389). (B) Final microarray layout: 24-plex array with 16 probes (summarized in the Table insert) printed in triplicate. Control antigens used in the microarray included 50 µg/mL of biotinylated human immunoglobulins G, A, and M (hIgG, hIgA, and hIgM, respectively; Rockland, Gilbertsville, PA, USA) and 132 µg/mL of biotinylated anti-human immunoglobulin G (anti-hIgG; Rockland) as well as in house derivatized NHS-ester-Cy3 (Thermo Scientific) biotinylated BSA (Cy3-BSA) at 40 µg/mL. Figure S2. PMID: 33925055.



Figure

Characterization of a Star635P-conjugated polyclonal antihuman IgM Fab. a Epifluorescence images of wild type and IgM-negative (IgMneg) Ramos B cells stained with a monovalent Star635P-conjugated Fab against human IgM (Fab-Star635P). Fluorescent images were acquired under the same conditions and equally scaled to allow a direct comparison. Transmission light inset in the image on the right-hand side shows the presence of IgMneg cells. Scale bars represent 10 µm. b Ca2+ mobilization analysis of wild type Ramos B cells stimulated (indicated by an arrow) with either polyclonal anti-IgM F(ab')2 (blue curve) or the Star635P-conjugated monovalent anti-IgM Fab (red curve). c Wild type Ramos B cells were stained with increasing concentrations of Fab-Star635P to establish the optimal concentration needed for saturated labeling of mIgM-BCRs on the cell surface. Dilutions ranged from 1:10 (0.1%) to 1:1000 (0.001%) from our stock of fluorescently-labeled Fab. After background correction regions of interest containing whole cells were manually selected and the average intensity of whole cells was calculated. Values represent the average of cellular 6 fluorescence intensities from >50 cells per concentration point. Error bars represent the standard error of the mean. The arrow indicates the final concentration that was used in the experiments shown. d STED images of single Fab-Star635P and Fab-Star635P mixed with a monomeric Ig µ heavy chain seeded on glass coverslips (scale bar 1µm). The bar graph shows the mean fluorescence intensity of single spots and error bars represent the standard error of the mean from eight independent experiments. e, f Confocal images of plasma membrane sheets stained with R18 (pseudocolored in green) and STED images showing the Fab-Star635P fluorescence signal derived from either untreated Ramos B cells (e) or cells that were BCR-activated with a monoclonal anti-IgM antibody (f). Membrane sheets were analyzed as described for Figure 1. Example histograms showing the fluorescence intensity distributions of Fab-Star635Pconjugated on coverslips (black curves, control) and mIgM-spots present in membrane sheets (red curves, mIgM-BCRs). Supplementary Figure 3. PMID: 30778055.

References

- Smith M et al. Age, Disease Severity and Ethnicity Influence Humoral Responses in a Multi-Ethnic COVID-19 Cohort. *Viruses.* (2021)
- Gomes de Castro, MA et al. Differential organization of tonic and chronic B cell antigen receptors in the plasma membrane. *Nature Communications* (2019)

Disclaimer

No test method can provide total assurance that the hepatitis B virus, hepatitis C virus, human immunodeficiency virus, or any other infectious agents are absent. Thus, all blood products, including purified proteins derived from human blood sources, should be handled at Biosafety Level 2 as recommended by the CDC\NIH manual entitled Biosafety in Microbiological and Biomedical Laboratories for potentially infectious human serum, blood specimens or proteins derived from same. Source material for the human blood product supplied to your facility has been tested for the detection of HIV antibody, Hepatitis B surface antigen, antibody to Hepatitis C, HIV 1 antigen(s), antibody to HTLV - I/II, and syphilis by FDA guidelines. All units were found to be non-reactive/negative for these tests. All human blood source material is collected in FDA licensed centers and is tested with FDA approved test kits.

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