Catalog # CD0-HF2H5



### **Synonym**

MS4A1,CD20,MS4A-1

#### Source

FITC-Labeled Human CD20 Full Length Protein, His Tag(CD0-HF2H5) is expressed from human 293 cells (HEK293). It contains AA Met 1 - Pro 297 (Accession # P11836-1).

# **Molecular Characterization**

Predicted N-terminus: Met 1

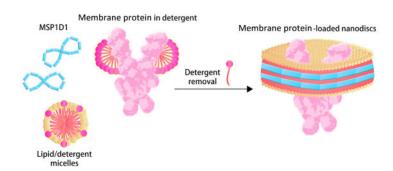
CD20(Met 1 - Pro 297) P11836-1

Poly-his

This protein carries a polyhistidine tag at the C-terminus.

The protein has a calculated MW of 35.2 kDa. The protein migrates as 26 kDa, 40 kDa and 70 kDa when calibrated against <u>Star Ribbon Pre-stained Protein</u> <u>Marker</u> under reducing (R) condition (SDS-PAGE) due to glycosylation.

Nanodiscs are a new class of model membranes that are being used to solubilize and study a range of integral membrane proteins and membrane-associated proteins. The Nanodisc bilayer is bounded by a membrane scaffold protein (MSP1D1) coat that confers enhanced stability and a narrow particle size distribution.



The nanodisc assembles from a mixture of full length membrane protein in detergent, phospholipid micelles and membrane scaffold protein(MSP1D1) upon removal of the detergent.

## Conjugate

### FITC

Excitation source: 488 nm spectral line, argon-ion laser

Excitation Wavelength: 488 nm

Emission Wavelength: 535 nm

# Labeling

The primary amines in the side chains of lysine residues and the N-terminus of the protein are conjugated with FITC using standard chemical labeling method. The residual FITC is removed by molecular sieve treatment during purification process.

## **Purity**

>85% as determined by SDS-PAGE.

### **Formulation**

Supplied as 0.2  $\mu$ m filtered solution in 50 mM HEPES, 150 mM NaCl, pH7.5 with trehalose as protectant.

Contact us for customized product form or formulation.

## **Shipping**

This product is supplied and shipped with dry ice, please inquire the shipping cost.

### **Storage**

Please protect from light and avoid repeated freeze-thaw cycles.

This product is stable after storage at:

- The product MUST be stored at -70°C or lower upon receipt;
- -70°C for 3 months under sterile conditions.
- \*The isotype control of empty/mock nanodisc (Cat. No. <u>APO-H51H3</u>) is sold separately and not included in protein, you can follow <u>this link</u> for product information.



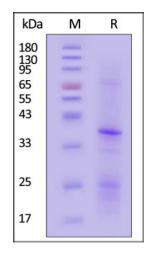
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### **Endotoxin**

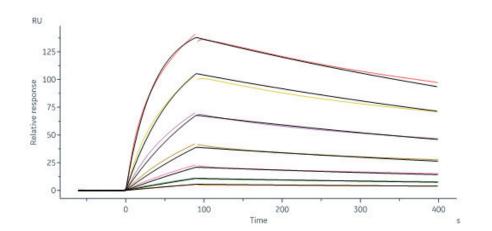
Less than 1.0 EU per µg by the LAL method.

### **SDS-PAGE**

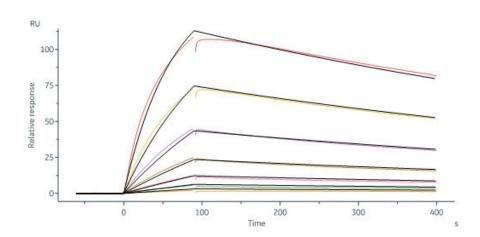


FITC-Labeled Human CD20 Full Length Protein, His Tag on SDS-PAGE under reducing (R) condition. The gel was stained with Coomassie Blue. The purity of the protein is greater than 85% (With <u>Star Ribbon Pre-stained Protein Marker</u>).

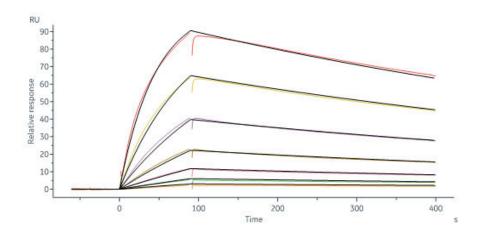
## **Bioactivity-SPR**



Obinutuzumab captured on Protein A Chip can bind FITC-Labeled Human CD20 Full Length Protein, His Tag (Cat. No. CD0-HF2H5) with an affinity constant of 23.4 nM as determined in a SPR assay (Biacore 8K) (QC tested).



Ofatumumab captured on Protein A Chip can bind FITC-Labeled Human CD20 Full Length Protein, His Tag (Cat. No. CD0-HF2H5) with an affinity



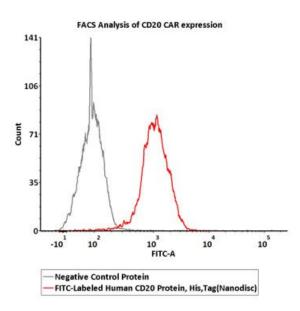
Rituximab captured on Protein A Chip can bind FITC-Labeled Human CD20 Full Length Protein, His Tag (Cat. No. CD0-HF2H5) with an affinity constant of 27.3 nM as determined in a SPR assay (Biacore 8K) (Routinely tested).

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constant of 37.3 nM as determined in a SPR assay (Biacore 8K) (Routinely tested).

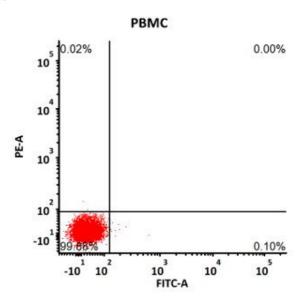
### **Bioactivity-FACS**

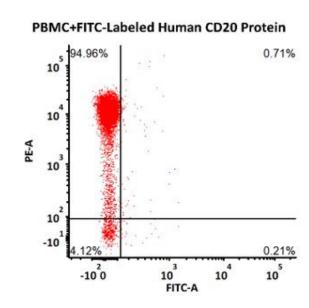


Flow cytometric analysis of Anti-CD20 CAR-293 cells staining with FITC-Labeled Human CD20 Full Length Protein, His Tag (Cat. No. CD0-HF2H5) at 1  $\mu$ g/mL (1  $\mu$ g/mL corresponds to labeling of 2.5e5 cells in a final volume of 100  $\mu$ L), compared with negative control protein. FITC signal was used to evaluate the binding activity (QC tested).

### **Evaluation of CAR expression**

FACS Analysis of Non-specific binding to PBMCs





Non-specificity of FITC-Labeled Human CD20 Full Length Protein, His Tag (Cat. No. CD0-HF2H5) binding to CD3+ cells present in human PBMC. 5e5 of human PBMCs were simultaneously stained with PE-labeled anti-CD3 antibody and FITC-Labeled Human CD20 Full Length Protein, His Tag (1 µg/mL corresponds to labeling of 5e5 cells in a final volume of 100 µL) and washed and then analyzed with FACS. Both FITC and PE positive signals was used to evaluate the non-specific binding activity to human CD3+ cells (QC tested).

### **Background**

B-lymphocyte antigen CD20 is also known as B-lymphocyte surface antigen B1, Leukocyte surface antigen Leu-16, Membrane-spanning 4-domains subfamily A member 1 and MS4A1, is an activated-glycosylated phosphoprotein expressed on the surface of all B-cells beginning at the pro-B phase (CD45R+, CD117+) and progressively increasing in concentration until maturity. CD20 is expressed on all stages of B cell development except the first and last; it is present from late pro-B cells through memory cells, but not on either early pro-B cells or plasma blasts and plasma cells. It is found on B-cell lymphomas, hairy cell leukemia, B-cell chronic



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lymphocytic leukemia, and melanoma cancer stem cells. The protein has no known natural ligand and its function is to enable optimal B-cell immune response, specifically against T-independent antigens. It is suspected that it acts as a calcium channel in the cell membrane. CD20 / MS4A1 is the target of the monoclonal antibodies (mAb) rituximab, Ibritumomab tiuxetan, and tositumomab, which are all active agents in the treatment of all B cell lymphomas and leukemias. Defects in CD20 / MS4A1 are the cause of immunodeficiency common variable type 5 (CVID5); also called antibody deficiency due to CD20 defect. CVID5 is a primary immunodeficiency characterized by antibody deficiency, hypogammaglobulinemia, recurrent bacterial infections and an inability to mount an antibody response to antigen.

## **Clinical and Translational Updates**

Please contact us via <u>TechSupport@acrobiosystems.com</u> if you have any question on this product.

