Catalog # CD9-H5251



#### Synonym

CD19,B4,CVID3,MGC12802

#### Source

Human CD19 (20-291), Fc Tag, premium grade(CD9-H5251) is expressed from human 293 cells (HEK293). It contains AA Pro 20 - Lys 291 (Accession # P15391-1).

#### Predicted N-terminus: Pro 20

It is produced under our rigorous quality control system that incorporates a comprehensive set of tests including sterility and endotoxin tests. Product performance is carefully validated and tested for compatibility for cell culture use or any other applications in the early preclinical stage. When ready to transition into later clinical phases, we also offer a custom GMP protein service that tailors to your needs. We will work with you to customize and develop a GMP-grade product in accordance with your requests that also meets the requirements for raw and ancillary materials use in cell manufacturing of cell-based therapies.

## **Molecular Characterization**

CD19(Pro 20 - Lys 291) Fc(Pro 100 - Lys 330) P15391-1 P01857

This protein carries a human IgG1 Fc tag at the C-terminus.

The protein has a calculated MW of 56.6 kDa. The protein migrates as 70 kDa when calibrated against <u>Star Ribbon Pre-stained Protein Marker</u> under reducing (R) condition (SDS-PAGE) due to glycosylation.

#### Endotoxin

Less than 0.01 EU per  $\mu$ g by the LAL method / rFC method.

## Sterility

Negative

## Mycoplasma

Negative.

### Purity

>90% as determined by SDS-PAGE.

>90% as determined by SEC-MALS.

#### Formulation

Lyophilized from 0.22  $\mu$ m filtered solution in PBS, pH7.4 with trehalose as protectant.

Contact us for customized product form or formulation.

#### Reconstitution

Please see Certificate of Analysis for specific instructions.

For best performance, we strongly recommend you to follow the reconstitution protocol provided in the CoA.

#### Storage

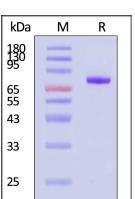
For long term storage, the product should be stored at lyophilized state at -20°C or lower.

Please avoid repeated freeze-thaw cycles.

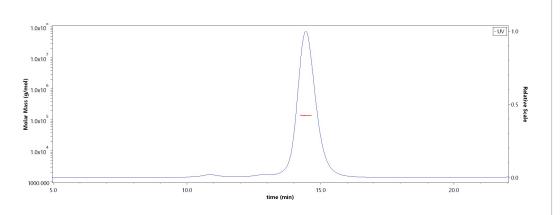
This product is stable after storage at:

- -20°C to -70°C for 12 months in lyophilized state;
- $-70^{\circ}$ C for 3 months under sterile conditions after reconstitution.

## **SDS-PAGE**



# SEC-MALS





Human CD19 (20-291), Fc Tag, premium grade on SDS-PAGE under reducing (R) condition. The gel was stained with Coomassie Blue. The purity of the protein is greater than 90% (With <u>Star Ribbon Pre-stained Protein Marker</u>).

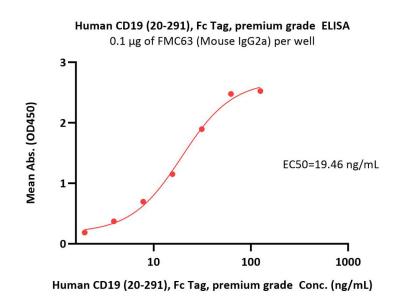
The purity of Human CD19 (20-291), Fc Tag, premium grade (Cat. No. CD9-H5251) is more than 90% and the molecular weight of this protein is around 130-160 kDa verified by SEC-MALS. <u>Report</u>

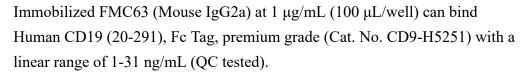


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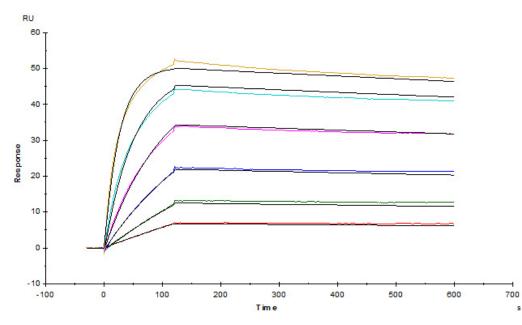
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## **Bioactivity-ELISA**



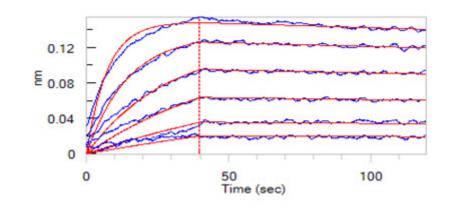


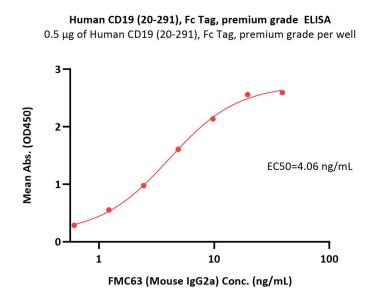
## **Bioactivity-SPR**



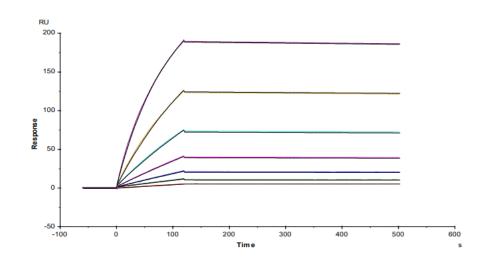
Human CD19 (20-291), Fc Tag, premium grade (Cat. No. CD9-H5251) captured on CM5 chip via Anti-Human lgG Fc antibodies surface, can bind FMC63 MAb (Mouse lgG2a) with an affinity constant of 0.17 nM as determined in a SPR assay (Biacore T200) (Routinely tested).

# **Bioactivity-BLI**





Immobilized Human CD19 (20-291), Fc Tag, premium grade (Cat. No. CD9-H5251) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind FMC63 (Mouse IgG2a) with a linear range of 0.6-5 ng/mL (Routinely tested).



Bispecific T cell Engager (CD3 X CD19) captured on CM5 chip, can bind Human CD19 (20-291), Fc Tag, premium grade (Cat. No. CD9-H5251) with an affinity constant of 0.93 nM as determined in a SPR assay (Biacore T200) (Routinely tested).





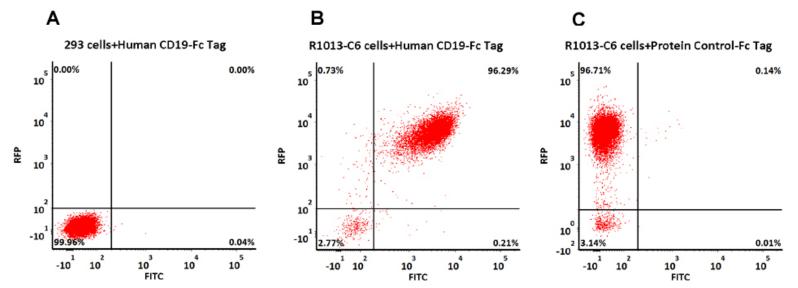
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Loaded Human CD19 (20-291), Fc Tag, premium grade (Cat. No. CD9-H5251) on AHC Biosensor, can bind FMC63 MAb (Mouse lgG2a) with an affinity constant of 0.483 nM as determined in BLI assay (ForteBio Octet Red96e) (Routinely tested).

## **Evaluation of CAR expression**

FACS Analysis of anti-CD19 CAR Expression



293 cells were transfected with FMC63-scFv and RFP tag. 2e5 of the cells were first stained with B. Human CD19 (20-291), Fc Tag, premium grade (Cat. No. CD9-H5251, 3 μg/mL) and C. Human Fc Tag Protein Control, followed by FITC-conjugated Anti-human IgG Fc Antibody. A. Non-transfected 293 cells and C. Human Fc Tag Protein Control. RFP was used to evaluate CAR (anti-CD19-scFv) expression and FITC was used to evaluate the binding activity of Human CD19 (20-291), Fc Tag, premium grade (Cat. No. CD9-H5251).

#### Background

B-lymphocyte antigen CD19 is also known as CD19 (Cluster of Differentiation 19), is a single-pass type I membrane protein which contains two Ig-like C2-type (immunoglobulin-like) domains. CD19 is expressed on follicular dendritic cells and B cells. In fact, it is present on B cells from earliest recognizable B-lineage cells during development to B-cell blasts but is lost on maturation to plasma cells. It primarily acts as a B cell co-receptor in conjunction with CD21 and CD81. Upon activation, the cytoplasmic tail of CD19 becomes phosphorylated, which leads to binding by Src-family kinases and recruitment of PI-3 kinase. As on T cells, several surface molecules form the antigen receptor and form a complex on B lymphocytes. The (almost) B cell-specific CD19 phosphoglycoprotein is one of these molecules. The others are CD21 and CD81. These surface immunoglobulin (sIg)-associated molecules facilitate signal transduction. On living B cells, anti-immunoglobulin antibody mimicking exogenous antigen causes CD19 to bind to sIg and internalize with it. The reverse process has not been demonstrated, suggesting that formation of this receptor complex is antigen-induced. This molecular association has been confirmed by chemical studies. Mutations in CD19 are associated with severe immunodeficiency syndromes characterized by diminished antibody production. CD19 has been shown to interact with: CD81, CD82, Complement receptor 2, and VAV2.



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