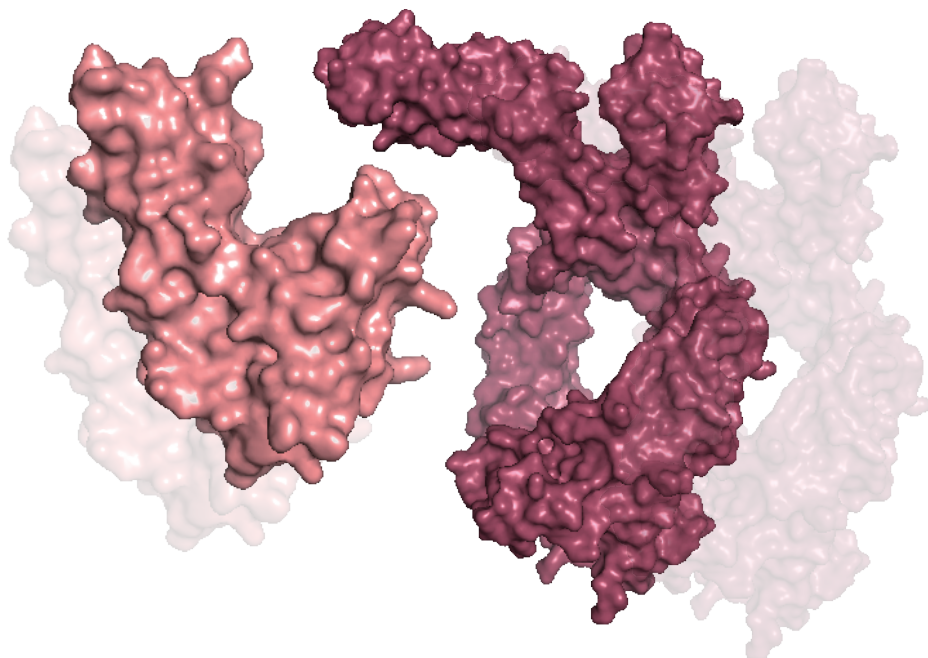




SPR-verified Fc Receptor Proteins

—— SPR Protocol Offered



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Introduction

The neonatal Fc receptor is an Fc receptor with similar structure to the MHC class I molecule. It forms a heterodimer protein with beta-2-microglobulin. It was first discovered in rodents as a receptor capable of transporting IgG across the epithelium of newborn's gut into the bloodstream. That's why it was later named as neonatal Fc receptor (FcRn). Further studies identified similar receptors in humans and other mammals.

The main function for FcRn is to extend the half-life of IgG and serum albumin by reducing lysosomal degradation in endothelial cells. As shown in Fig. 1, IgG and serum albumin are bound by FcRn under acidic pH (<6.5), and recycled to the cell surface, where they are released to the blood at the neutral pH (>7.0). This mechanism protects IgG and serum albumin from lysosomal degradation.

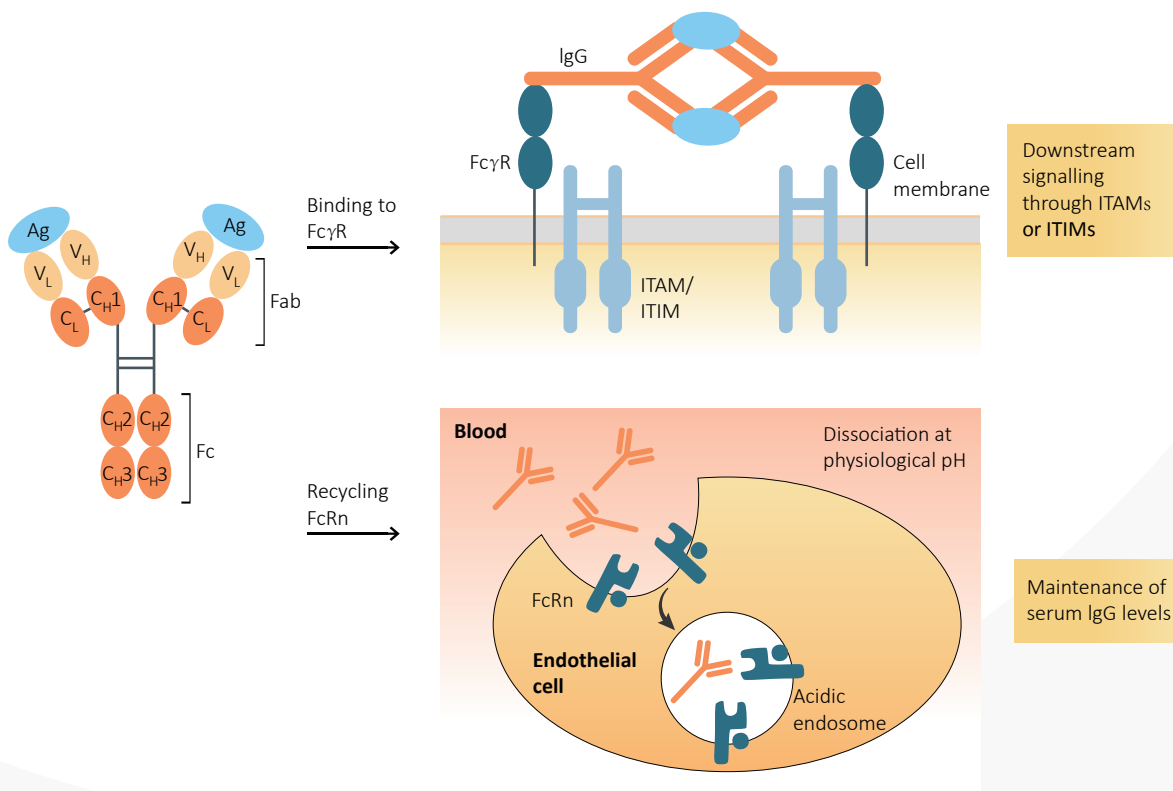


Fig. 1. Function of Fc receptor.

In addition to FcRn, three classes of Fc gamma receptors (Fc γ Rs) can also interact with IgG in humans. These receptors are differentially expressed by monocytes, macrophages, neutrophils, eosinophils, B and T cells, dendritic cells, natural killer cells, and platelets. Human Fc γ Rs bind IgG with different affinities ranging from low ($>10^{-7}$ M, Fc γ RII, or CD32), medium ($<10^{-7}$ M, Fc γ RIII, or CD16), to high (10^{-8} – 10^{-9} M, Fc γ RI, or CD64). Different IgG isotypes also demonstrate different binding affinities to these receptors. The interaction between IgG and Fc-gamma receptors mediates a variety of cellular function in particular antibody-dependent cell-mediated cytotoxicity (ADCC).

Polymorphisms of the Fc γ Rs are common. It can affect mAb binding. Some Fc γ R polymorphism has been linked with clinical outcomes. For example, a clinical study of rituximab (anti-CD20) in the treatment of non-Hodgkin's lymphoma has revealed a higher response rate among patients with Fc γ RIIIa(V176) homozygous, as compared to those with F176/V176 heterozygous and F176 homozygous.

Another example is a study on the side-effects of OKT3, the mouse monoclonal anti-CD3 antibody. OKT3 is used as an immunosuppressive agent to prevent rejection of solid-organ transplants. It was shown that the severity of this 'first-dose reaction' was correlated with the presence of the Fc γ RIIa(R167) allotype, which interacts with mIgG1.

The efficacy of a therapeutic antibodies depends on its interaction with FcRn and Fc-gamma receptors. Therefore, candidates must be tested against a panel of receptors during antibody engineering. ACROBiosystems offers a comprehensive collection of recombinant Fc receptor proteins, including their common variants, to help expedite your research program.

Key Features of Product

■ High purity

To meet the high purity requirement of pharmaceutical applications, our production team perform both SDS-PAGE and HPLC analyses to the Fc receptors for QC purposes. It's noteworthy that it's essential to use monomeric Fc receptor, which represents its natural state. The formation of oligomers during production may result in artificially enhanced binding interaction due to avidity effect. To avoid such situation, we have established strict internal control standards of monomer purity by HPLC testing. Only those batches meeting all purity requirements are released.

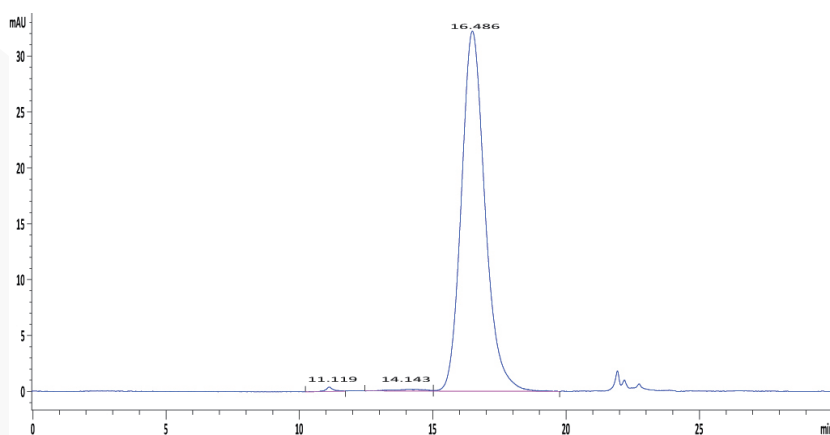


Fig. 2. The purity of Human Fc gamma RIIA / CD32a (R167) Protein (Cat. No. CDA-H5221) is greater than 95% as determined in a HPLC analysis.

■ Bioactivity validated by SPR

The binding affinity between two molecules can be determined by many methods. However, the studies of Fc interaction are often done by SPR, because traditional ELISA is not appropriate for low affinity receptors such as CD16 and CD32.

To assure our Fc receptors deliver expected performance, our QC team use Biacore platform to test our products against reference antibodies. **All protocols for SPR are available for free.**

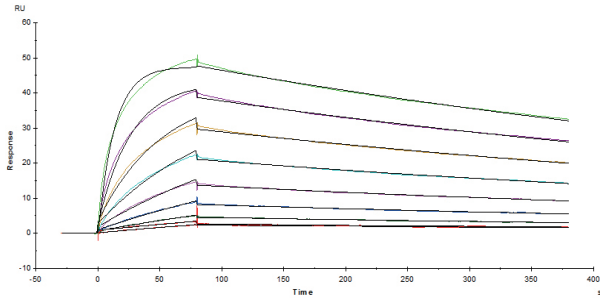


Fig. 3. Immobilized Human Fc gamma RI / CD64, His Tag (Cat. No. FCA-H52H2) on CM5 Chip via anti-His antibody, can bind Herceptin with an affinity constant of 5.45 nM as determined in a SPR assay (Biacore T200).

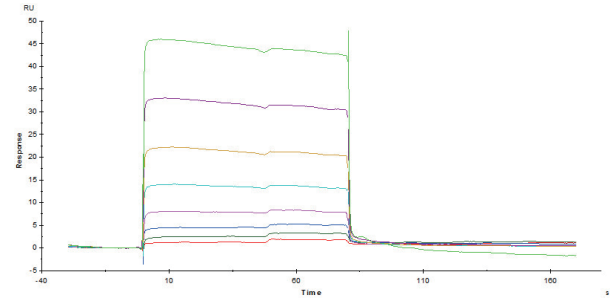


Fig. 4. Immobilized Human CD32a (R167) (Cat. No. CDA-H5221) on CM5 Chip via anti-His antibody, can bind Rituximab with an affinity constant of 3.12 μ M as determined in SPR assay (Biacore T200).

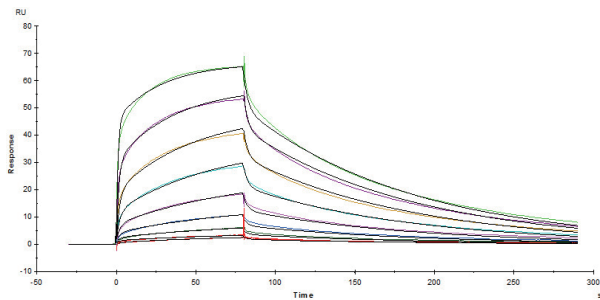


Fig. 5. Immobilized Human CD16a (V176) Protein (Cat. No. CD8-H52H4) on CM5 Chip via anti-His antibody, can bind Herceptin with an affinity constant of 163 nM as determined in SPR assay (Biacore T200).

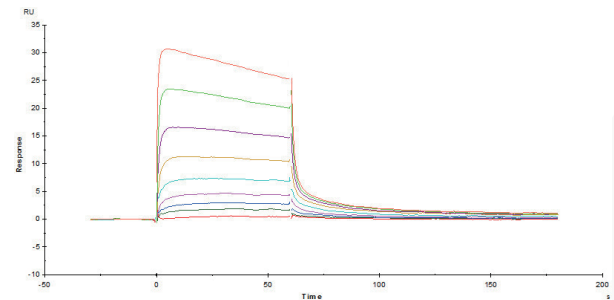


Fig. 6. Immobilized Human FcRn / FCGR2 & B2M Heterodimer Protein (Cat. No. FCM-H5286) on CM5 Chip via anti-His antibody, can bind Herceptin with an affinity constant of 0.489 μ M as determined in a SPR assay (Biacore T200).

■ High batch-to-batch consistency

We routinely apply rigorous quality control measures to ensure consistent performance of our product. As shown below, the batch variation among the tested samples is negligible.

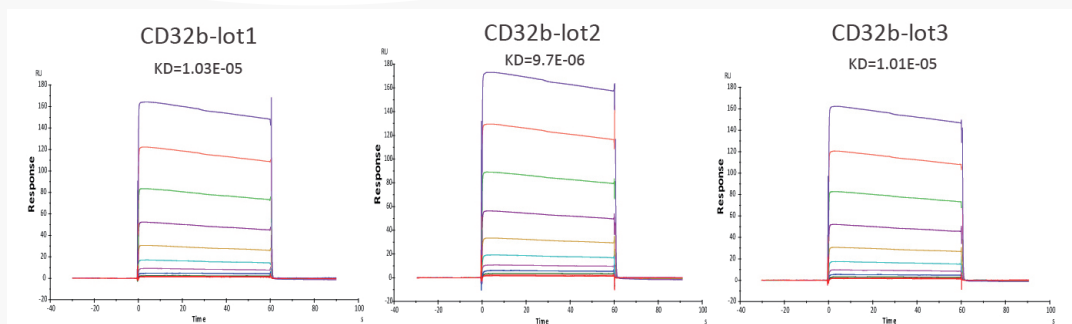


Fig. 7. Immobilized Human Fc gamma RIIB / CD32b Protein (Cat. No. CDB-H5228) on CM5 Chip via anti-His antibody, can bind Rituximab with an affinity constant of 10 μ M as determined in a SPR assay (Biacore T200).

We have compared the performance of different batches of CD32b (CDB-H5228) in the same assay, and the software analysis showed that the similarity score was very high, meaning high batch-to-batch consistency.

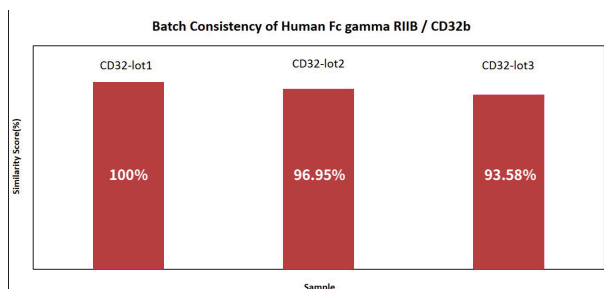


Fig. 8. Batch consistency of Human Fc gamma RIIB / CD32b (Cat. No. CDB-H5228). The Similarity for different batches is more than 90%.

SPR Method	Ligand	Ligand Cat. No.	Analyte	KD (M)	Similarity Score (%)	KD RSD (%)
His Capture	CD32b-Lot1	CDB-H5228	Rituximab	1.03E-05	100	5
	CD32b-Lot2	CDB-H5228	Rituximab	9.7E-06	96.95	
	CD32b-Lot3	CDB-H5228	Rituximab	1.01E-05	93.58	

Biotinylated or Other Species Fc Receptors Available

The use of biotin labeling can make your assay development much easier. We offer a variety of ready-to-use biotinylated Fc receptors. These proteins are produced using our in-house developed labeling techniques, which confers high bioactivity and minimal batch-to-batch variation. In addition, we have developed a series of Fc receptors of other species, which are suitable for the screening of non-humanized antibodies or the species cross reaction.

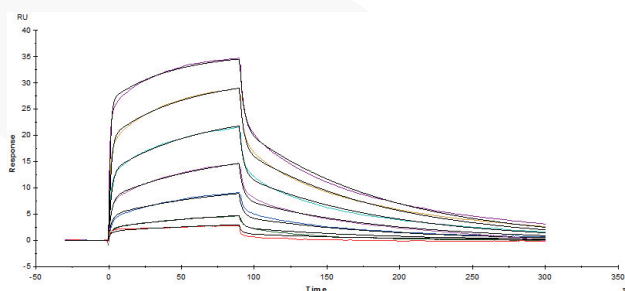


Fig. 9. Biotinylated Human CD16a (V176), His Tag, Avi Tag (Cat. No. CDA-H82E9) captured on Biotin CAP- Series S Sensor Chip can bind Rituximab with an affinity constant of 0.261 μM as determined in a SPR assay (Biacore T200).

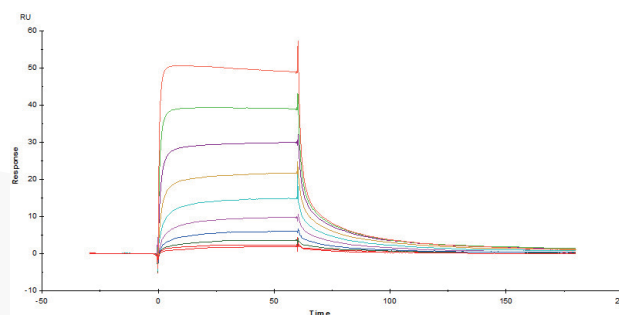


Fig. 10. Immobilized Cynomolgus / Rhesus macaque FcRn Protein (Cat. No. FCM-C5284) on CM5 Chip via anti-His antibody, can bind Herceptin with an affinity constant of 0.403 μM as determined in a SPR assay (Biacore T200).

Product List

Molecule	Cat.No.	Species	Structure	Size	Feature
FcRn (FCGRT & B2M)	FCM-H5286	Human	FcGRT(24-297) His B2M(21-119) Strep II	50ug,1mg	SPR verified
FcRn (FCGRT & B2M) (Biotin-labeled)	FCM-H8286	Human	FcGRT(24-297) His B2M(21-119) Strep II	25ug,200ug	
FcRn (FCGRT & B2M) (Biotin-labeled)	FCM-H82W4	Human	FcGRT(24-297) His Avi B2M(21-119) Strep II	25ug,200ug	
FcRn (FCGRT & B2M)	FCM-C5284	Cynomolgus / Rhesus macaque	FcGRT(24-297) His B2M(21-119) Strep II	50ug,1mg	SPR verified
FcRn (FCGRT & B2M) (Biotin-labeled)	FCM-C82W5	Cynomolgus / Rhesus macaque	FcGRT(24-297) Avi His B2M(21-119) Strep II	25ug,200ug	
FcRn (FCGRT & B2M)	FCM-M52W2	Mouse	FcGRT(22-297) His B2M(21-119) Twin-Strep	50ug,1mg	
FcRn (FCGRT & B2M) (Biotin-labeled)	FCM-M82W6	Mouse	FcGRT(22-297) Avi His B2M(21-119) Strep II	25ug,200ug	
FcRn (FCGRT & B2M)	FCM-R5287	Rat	FcGRT(23-298) His B2M(21-119) Strep II	50ug,1mg	
FcRn (FCGRT & B2M) (Biotin-labeled)	FCM-R82W7	Rat	FcGRT(23-298) Avi His B2M(21-119) Strep II	25ug,200ug	
FcRn (FCGRT & B2M) (Biotin-labeled)	FCN-B82W3	Bovine	FcGRT(24-298) His Avi B2M(21-118)	25ug,200ug	
FcRn (FCGRT & B2M) (Biotin-labeled)	FCN-F82W3	Feline	FcGRT(24-297) His Avi B2M(21-118)	25ug,200ug	
FcRn (FCGRT & B2M)	FCM-P5280	Porcine	FcGRT(16-289) His B2M(21-118) Strep II	50ug,1mg	

Product List

Molecule	Cat.No.	Species	Structure	Size	Feature
Fc gamma RIIIA / CD16a	CDA-H5220 HOT	Human	F176 CD16a (17-208) His	100ug,1mg	SPR verified
Fc gamma RIIIA / CD16a (Biotin-labeled)	CDA-H82E8	Human	F176 CD16a (17-208) Avi His	25ug,200ug	SPR verified
Fc gamma RIIIA / CD16a	CD8-H52H4 HOT	Human	V176 CD16a (17-208) His	100ug,1mg	SPR verified
Fc gamma RIIIA / CD16a (Biotin-labeled)	CDA-H82E9	Human	V176 CD16a (17-208) Avi His	25ug,200ug	SPR verified
Fc gamma RIIIB / CD16b (NA1)	CDB-H5227	Human	CD16b (NA1) (17-200) His	100ug,1mg	SPR verified
Fc gamma RIIIB / CD16b (NA1) (Biotin-labeled)	CDB-H82E4	Human	CD16b (NA1) (17-200) His Avi	25ug,200ug	SPR verified
Fc gamma RIIIB / CD16b (NA2)	CDB-H5222	Human	CD16b (NA2) (17-200) His	100ug,1mg	SPR verified
Fc gamma RIIIB / CD16b (NA2) (Biotin-labeled)	CDB-H82Ea NEW	Human	CD16b (NA2) (17-200) His Avi	25ug,200ug	SPR verified
Fc gamma RIII / CD16	FC6-C52H9 NEW	Cynomolgus	CD16 (17-208) His	100ug,1mg	
Fc gamma RIII / CD16 (Biotin-labeled)	FC6-C82E0 NEW	Cynomolgus	CD16 (17-208) His Avi	25ug,200ug	
Fc gamma RIII / CD16	CDA-M52H8	Mouse	CD16 (32-215) His	100ug	
Fc gamma RIIA / CD32a	CD1-H5223 HOT	Human	H167 CD32a (36-218) His	100ug,1mg	SPR verified

Product List

Molecule	Cat.No.	Species	Structure	Size	Feature
Fc gamma RIIA / CD32a (Biotin-labeled)	CDA-H82E6	Human	^{H167} CD32a (36-218) Avi His	25ug,200ug	SPR verified
Fc gamma RIIA / CD32a	CDA-H5221	Human	^{R167} CD32a (36-218) His	100ug,1mg	SPR verified
Fc gamma RIIA / CD32a (Biotin-labeled)	CDA-H82E7	Human	^{R167} CD32a (36-218) Avi His	25ug,200ug	SPR verified
Fc gamma RIIB / CD32b	CDB-H5228	Human	CD32b (46-217) His	100ug,1mg	SPR verified
Fc gamma RIIB / CD32b (Biotin-labeled)	CDB-H82E0	Human	CD32b (46-217) Avi His	25ug,200ug	SPR verified
Fc gamma RIIB / CD32b	CDB-M52H7	Mouse	CD32b (40-217) His	100ug,1mg	SPR verified
Fc gamma RIIB / CD32b (Biotin-labeled)	CDB-M82E8	Mouse	CD32b (40-217) Avi His	25ug,200ug	SPR verified
Fc gamma RI / CD64	FCA-H52H2	Human	CD64 (16-288) His	100ug,1mg	SPR verified
Fc gamma RI / CD64 (Biotin-labeled)	FCA-H82E8	Human	CD64 (16-288) His Avi	25ug,200ug	SPR verified
Fc gamma RI / CD64	CD4-M5227	Mouse	CD64 (25-297) His	100ug,1mg	
CD23	CD3-H5249	Human	His CD23 (48-321)	100ug,1mg	
CD23 (Biotin-labeled)	CD3-H82Q5	Human	His Avi CD23 (48-321)	25ug,200ug	

TIM-3 **CTLA-4** **4-1BB**
Immune Checkpoint
Biotinylated **HER2**
B7-H4 TIGIT 4-1BB Ligand
FCRn **CD40** **GITR**
PD-L2 **HER2**
DNAM-1 **B7-1**
TIM-3 **FCRn**
TNF- alpha **LAG-3**
CD19 **OX40** Ligand **B7-H2**
PD-1 **CD47** **PCSK9**
Immune Checkpoint
Biotin-labeled
VEGF165 **CD48** **PD-L1**
FcRn **HER2** **ICOS** **CD27** **CD48** **Checkpoint**
B7-H4 **BTLA** **CTLA-4**
TIM-3 **CTLA-4**
Biotin-labeled
HER2 **PD-L2** **B7-H4** **DNAM-1**
VEGF165 **FcRn** **PD-L1**
BTLA **CTLA-4**
DNAM-1 **PD-L2** **FCRn**
PD-L2 **PD-L2**
VEGF165 **4-1BB** **PCSK9** **Immune Checkpoint**
GITR Ligand **Biotinylated** **PD-1**
TNF-alpha **PD-L2**