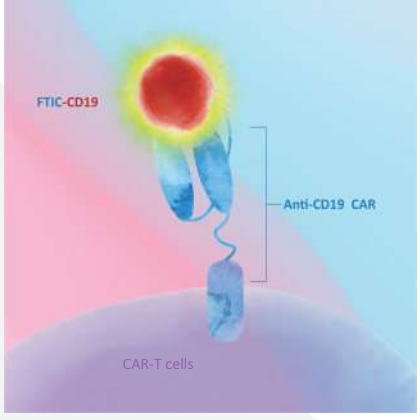
High Affinity CD19
Designed for Detection of Anti-CD19 CAR Expression

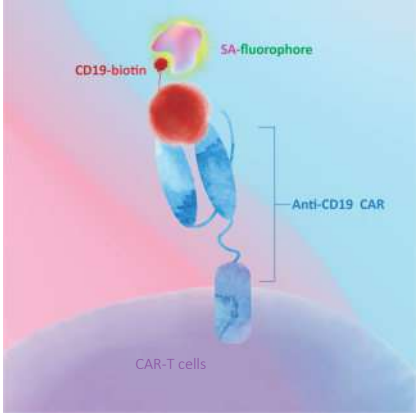
At present, CD19 is still the hottest target of CAR-T cell therapy. According to ClinicalTrials.gov, more than 600 CAR T cell clinical trials have been initiated so far, most of which aim to treat lymphoma or leukemia patients using CD19-specific CARs.

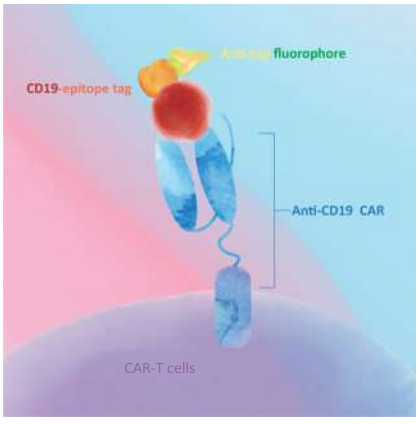
CAR-positive T cell is critical to the anti-tumor activity of anti-CD19 CAR T cell product. The percentage of CAR-positive T cells within the product is often measured by flow cytometry, using protein L, anti-Fab antibodies or CD19 antigen as detection antibodies. Among these common choices, CD19 antigen is widely considered to be the best option. Because it offers high specificity and minimal background staining.

ACROBiosystems has developed an extensive collection of CD19 antigen products to support anti-CD19 CAR T investigations, including fluorescent-labeled CD19 and biotinylated CD19 that are uniquely suitable for evaluation of anti-CD19 CAR expression.

Different CAR Detection Strategies and Product Design





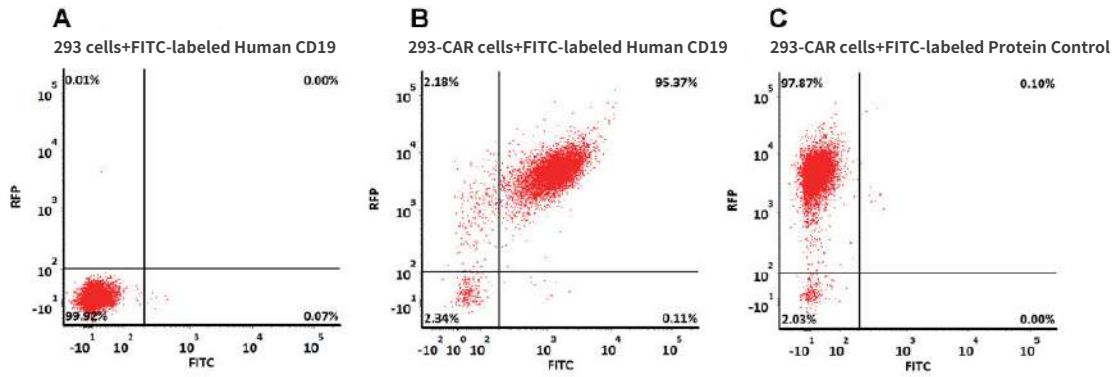


Direct Detection

CD19 is pre-labeled with FITC. Processing time can be reduced by the use of direct-labeled CD19 protein. Non-specific reaction of a secondary antibody is eliminated.

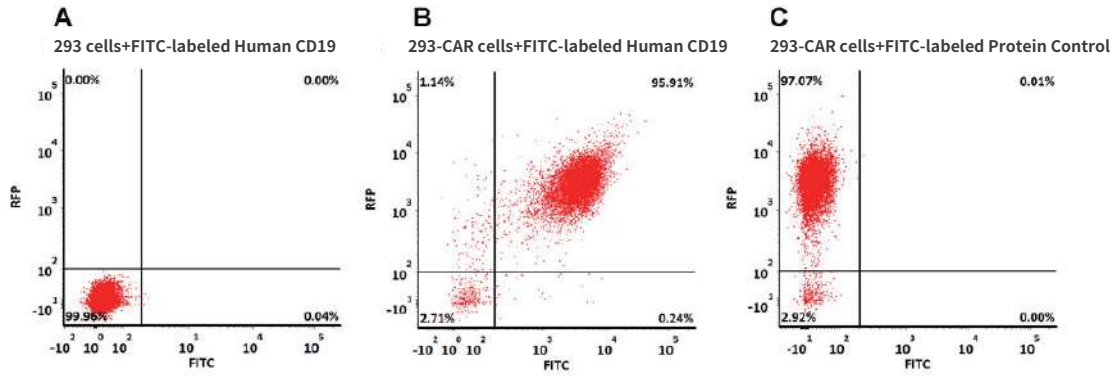
Cat. No.	Product Description	Structure
CD9-HF2H2	FITC-labeled Human CD19 (20-291) Protein	CD19(Pro 20-Lys 291) P15391-1 | Poly-his
CD9-HF251	FITC-labeled Human CD19 (20-291) Protein, Fc Tag	CD19(Pro 20-Lys 291) P15391-1 | Fc(Pro 100-Lys 330 P01857

CAR Detection by FITC-labeled Human CD19, His Tag



293 cells were transfected with anti-CD19-scFv and RFP tag. 2e5 of the cells were stained with B. FITC-labeled Human CD19 (20-291) (Cat. No. CD9-HF2H2, 10 μg/ml) and C. FITC-labeled protein control. A. Non-transfected 293 cells and C. FITC-labeled protein control were used as negative control. RFP was used to evaluate CAR (anti-CD19-scFv) expression and FITC was used to evaluate the binding activity of FITC-labeled Human CD19 (20-291) (Cat. No. CD9-HF2H2).

CAR Detection by FITC-labeled Human CD19, Fc Tag



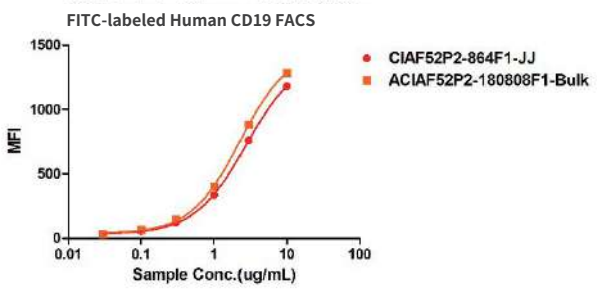
293 cells were transfected with anti-CD19-scFv and RFP tag. 2e5 of the cells were stained with B. FITC-labeled Human CD19 (20-291), Fc Tag (Cat. No. CD9-HF251, 10 μg/ml) and C. FITC-labeled protein control. A. Non-transfected 293 cells and C. FITC-labeled protein control were used as negative control. RFP was used to evaluate CAR (anti-CD19-scFv) expression and FITC was used to evaluate the binding activity of FITC-labeled Human CD19 (20-291), Fc Tag (Cat. No. CD9-HF251).

Competitive Advantage over Other Vendors

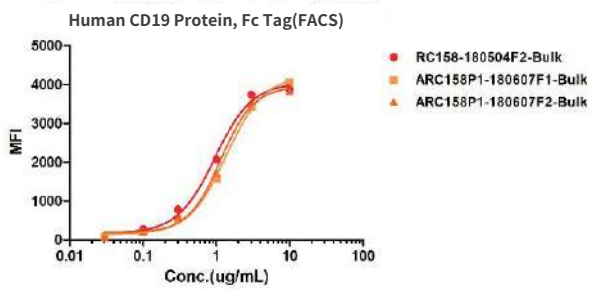
Binding activity of FITC-labeled Human CD19, His Tag from two different vendors were evaluated in the above flow cytometry analysis against anti-CD19-CAR-293 cells. The result showed that ACRO's FITC-labeled Human CD19, His Tag has a much higher binding activity than another vendor's.

Binding activity of FITC-labeled Human CD19, His Tag from two different vendors were evaluated in the above ELISA analysis against FMC63 MAb. The result showed that ACRO's FITC-labeled Human CD19, His Tag has a much higher binding activity than another vendor's.

Well Controlled Lot Consistency of FITC-labeled CD19

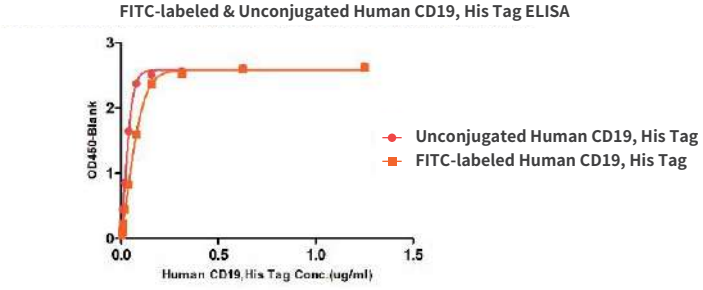


Binding activity of two different lots of FITC-labeled Human CD19, His Tag were evaluated in the above flow cytometry analysis against anti-CD19-CAR-293 cells. The result showed that the batch variation among the tested samples is negligible.

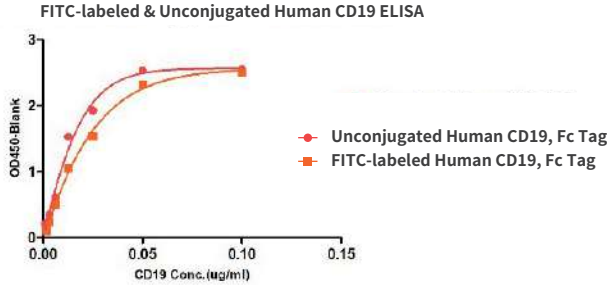


Binding activity of three different lots of FITC-labeled Human CD19, Fc Tag were evaluated in the above flow cytometry analysis against anti-CD19-CAR-293 cells. The result showed that the batch variation among the tested samples is negligible.

Consistent Binding Ability Before and After FITC Conjugation



Binding activity of the Human CD19, His Tag before and after FITC labeling were evaluated in the above ELISA analysis. The result showed that the FITC-labeled and Unconjugated Human CD19, His Tag have almost the same binding activity.



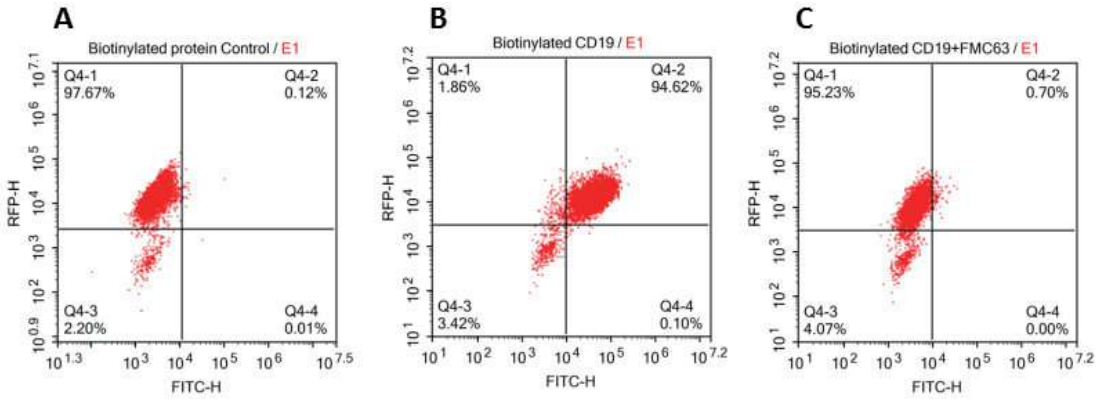
Binding activity of the Human CD19, Fc Tag before and after FITC labeling were evaluated in the above ELISA analysis. The result showed that the FITC-labeled and Unconjugated Human CD19, Fc Tag have almost the same binding activity.

Biotin-streptavidin Based Detection

CD19 is pre-labeled with biotin and detected by labeled streptavidin (the biotin-avidin complex). Streptavidin labeled with fluorochromes can bind biotinylated proteins with a high degree of affinity and specificity, amplifying the signal and improving the detection sensitivity and specificity.

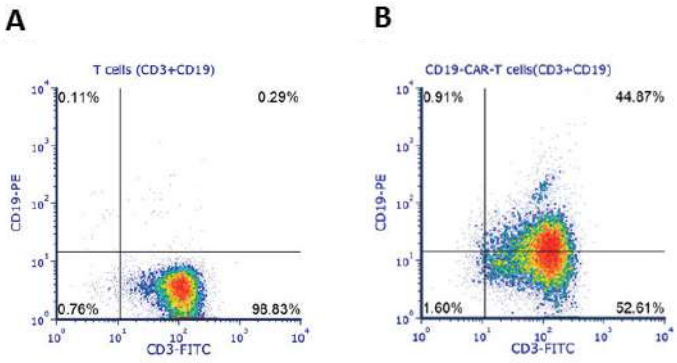
Cat. No.	Product Description	Structure
CD9-H8259	Biotinylated Human CD19, Fc Tag, ultra sensitivity (primary amine labeling)	CD19(Pro 20-Lys 291) P15391-1 | Fc(Pro 100-Lys 330 P01857

Indirect Detection by Biotinylated Human CD19, Fc Tag



293 cells were transfected with FCM63-scFv and RFP tag . 2e5 of the cells were first incubated with A. Biotinylated protein control. B. Recombinant biotinylated human CD19 (Cat. No. CD9-H8259, 10ug/ml) . C. Recombinant biotinylated human CD19 (Cat. No. CD9-H8259, 10ug/ml) and FMC63(Mouse anti-CD19 antibody). FITC Streptavidin was used to analyse with FACS. RFP was used to evaluate CAR(FMC63-scFv) expression and FITC was used to evaluate the binding activity of recombinant biotinylated human CD19 (Cat. No. CD9-H8259).

Case Study on Real CART Cells



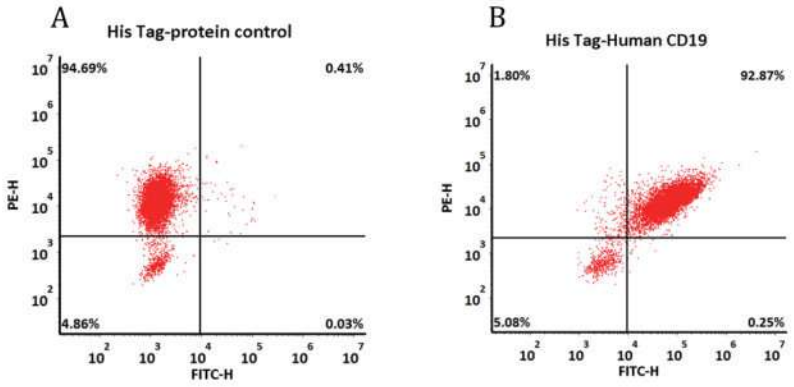
Human T cells were lentivirally transduced with anti-CD19 CAR and cultured for 11 days. Eleven days post-transduction, 1e6 cells were stained for the expression of CD3 and anti-CD19 CAR with FITC anti-human CD3 antibody and biotinylated human CD19 (Cat. No. CD9-H8259) followed by PE-conjugated streptavidin, respectively. A. Non-transduced T cells were used as a control for gating of CAR expression. (Data are kindly provided by Beijing Bowei Huaen Medical Technology Co. Ltd.)

Indirect Detection

CD19 is designed to carry a specific tag and detected using a secondary antibody (anti-epitope tag antibody) labeled with a fluorophore. Non-specific reaction of a secondary antibody may occur.

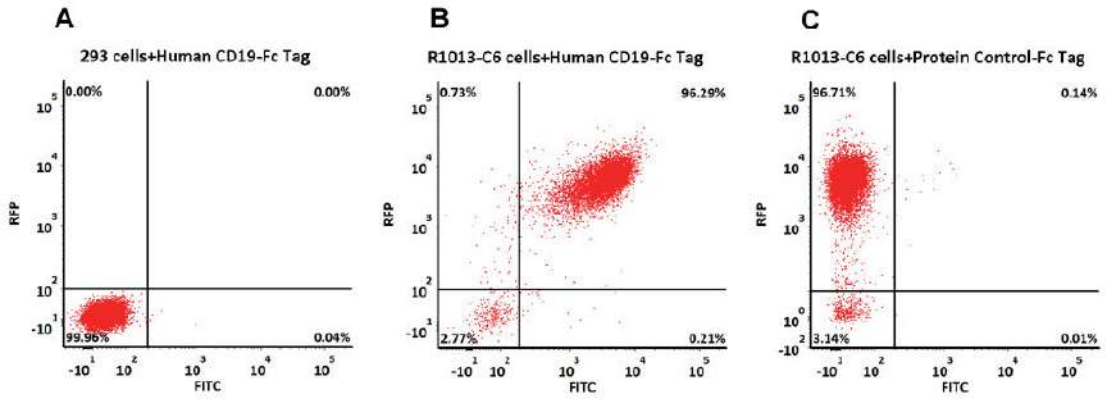
Cat. No.	Product Description	Structure
CD9-H52H2	Human CD19 (20-291) Protein, His Tag	CD19(Pro 20-Lys 291) P15391-1 | Poly-his
CD9-H5251	Human CD19 (20-291) Protein, Fc Tag, low endotoxin (Super affinity)	CD19(Pro 20-Lys 291) P15391-1 | Fc(Pro 100-Lys 330 P01857

Indirect Detection by Human CD19, His Tag



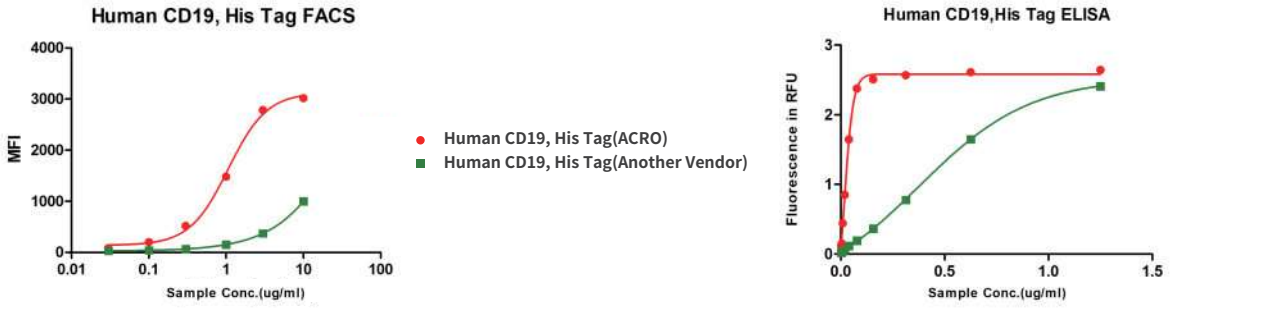
293 cells were transfected with FMC63-scFv and RFP tag. 2e5 of the cells were first incubated with A. His Tag-protein control. B. Recombinant human CD19, His Tag (Cat. No. CD9-H52H2, 10 μg/ml). The FITC Anti-6xHis tag antibody was used to analyse with FACS. RFP was used to evaluate CAR(FMC63-scFv) expression and FITC was used to evaluate the binding activity of recombinant human CD19, His Tag (Cat. No. CD9-H52H2).

Indirect Detection by Human CD19, Fc Tag



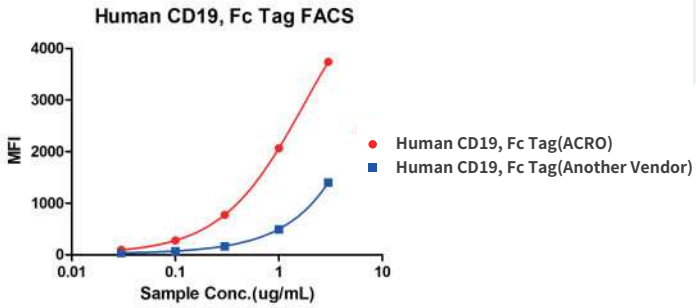
293 cells were transfected with FMC63-scFv and RFP tag. 2e5 of the cells were first stained with B. Human CD19 (20-291) Protein, Fc Tag, low endotoxin (Super affinity) (Cat. No. CD9-H5251, 3 μg/ml) and C. Human Fc Tag Protein Control, followed by FITC-conjugated Anti-human IgG Fc Antibody. A. Non-transfected 293 cells and C. Human Fc Tag Protein Control were used as negative 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) Protein, Fc Tag, low endotoxin (Super affinity) (Cat. No. CD9-H5251).

Higher Binding Affinity than Competitive Products

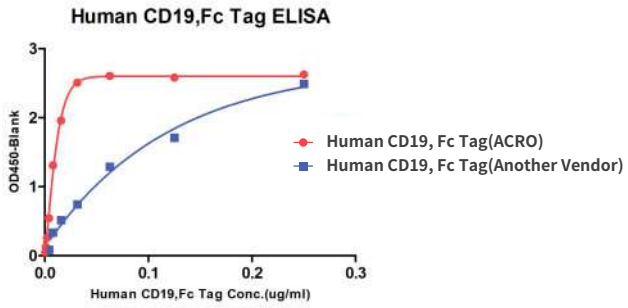


Binding activity of Human CD19, His Tag from two different vendors were evaluated in the above flow cytometry analysis against anti-CD19-CAR-293 cells. The result showed that ACRO's Human CD19, His Tag has a higher binding activity than another vendor's.

Binding activity of Human CD19, His Tag from two different vendors were evaluated in the above ELISA analysis against FMC63 MAb. The result showed that ACRO's Human CD19, His Tag has a higher binding activity than another vendor's.

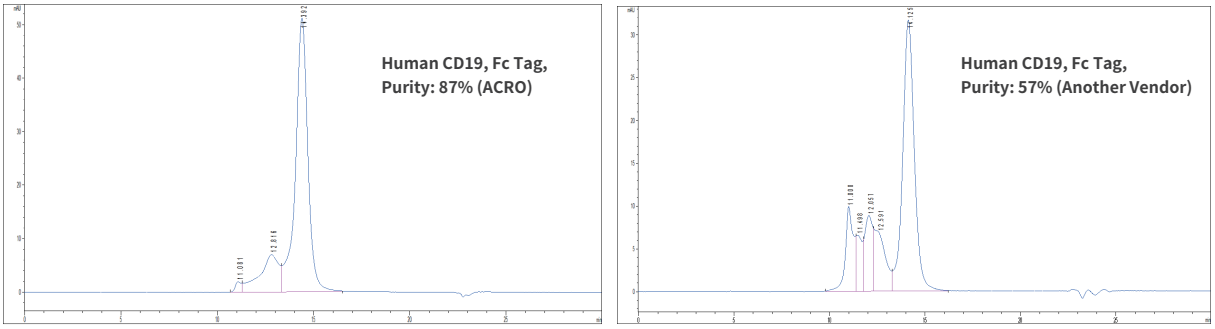


Binding activity of Human CD19, Fc Tag from two different vendors were evaluated in the above flow cytometry analysis against anti-CD19-CAR-293 cells. The result showed that ACRO's Human CD19, Fc Tag has a higher binding activity than another vendor's.



Binding activity of Human CD19, Fc Tag from two different vendors were evaluated in the above ELISA analysis against FMC63 MAb. The result showed that ACRO's Human CD19, Fc Tag has a higher binding activity than another vendor's.

Higher Purity than Competitive Products

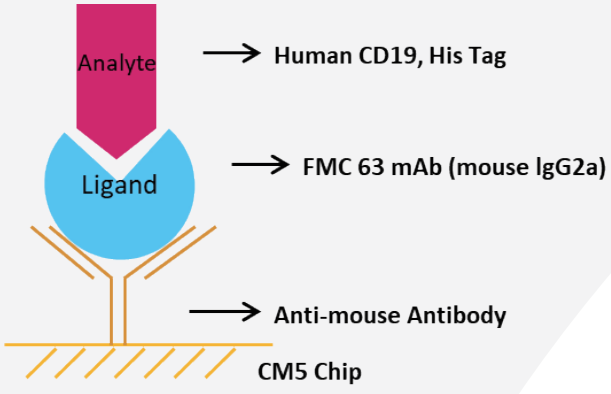


The purity of Human CD19, Fc Tag from two different vendors were determined in HPLC analysis. The result showed that the purity of ACRO's Human CD19, Fc Tag is greater than 87%, while the purity of another vendor's Human CD19, Fc Tag is greater than 57%.

Better Performance in SPR Studies than Competitive Products

Comparative analysis of rhCD19-his from ACRO and another vendor for affinity to FMC63 by SPR

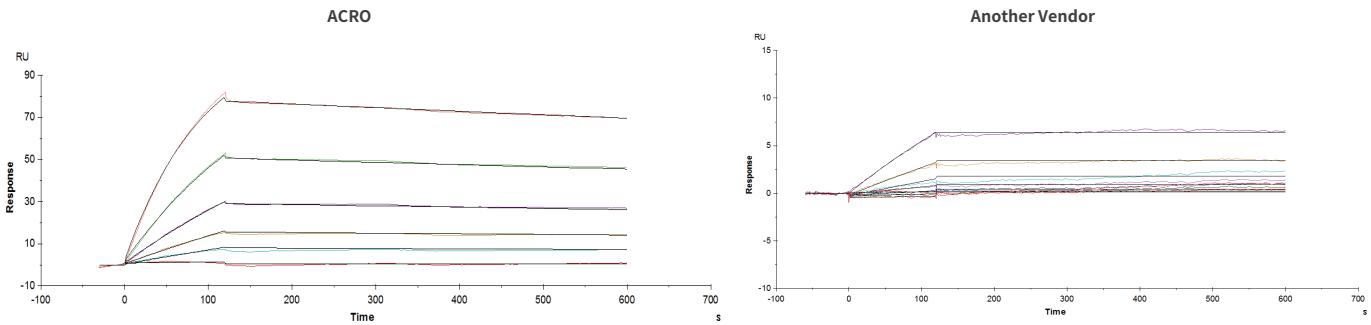
>>>Experimental Design



>>>Brief Protocol

- Chip Preparation: The sensor chip CM5 was activated using EDC/NHS. Anti-mouse antibody was immobilized on the CM5 sensor chip.
- Ligand Capturing: Capture FMC63 mAb to the CM5 chip with immobilized anti-mouse antibody.
- Analysis: A series of Human CD19, His Tag concentrations including 0nM, 5.86nM, 11.72nM, 23.44nM, 46.88nM, 93.75nM, 187.5nM and 375nM were injected consecutively, each with a contact time of 120 seconds and a dissociation time of 480 seconds.
- Regeneration: The chip was regenerated using 10 mM Glycine-HCl buffer at PH1.7.

>>>Experimental Results



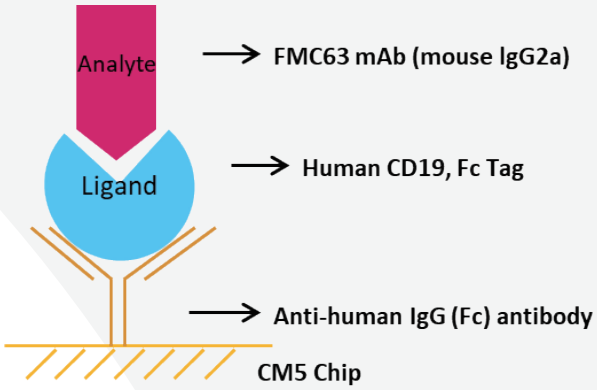
Product	Vendor	Method	Ligand	Capture level	Analyte Conc.	ka (1/Ms)	kd (1/s)	KD(M)	Rmax (RU)	% Ligand Bound	RU (375nM)
Human CD19, His Tag (Cat.No. CD9-H52H2)	ACRO	Mouse Antibody capture	FMC63	477 RU	hCD19 his 12-0.375ug/ml (240-7.5nM)	1.02E+05	3.00E-04	2.95E-09	91.2	28.7 %	85
Human CD19, His Tag	Another Vendor					N.A.	N.A.	N.A.	29.6	9.3%	6

>>>Conclusions

ACRO's rhCD19-His exhibited better performance in SPR assay compared to that of the counterpart from another vendor under the same conditions. The affinity constant (KD value) of ACRO's rhCD19-His to FMC63 was 2.95 nM, which was consistent with the published data (Nicholson, Ian C. et al.).

Comparative analysis of rhCD19-Fc from ACRO and another vendor for affinity to FMC63 by SPR

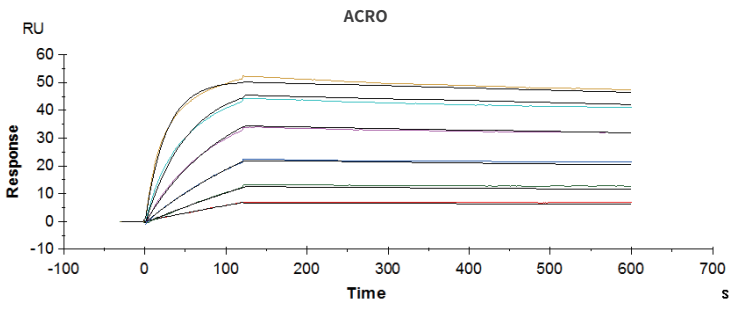
>>>Experimental Design

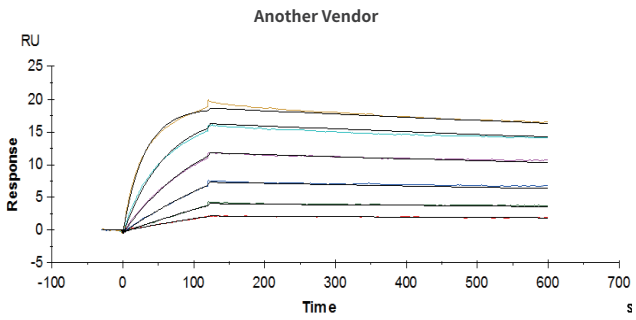


>>>Brief Protocol

- Chip Preparation: The sensor chip CM5 was activated using EDC/NHS. Anti-human IgG (Fc) antibody was immobilized on the CM5 sensor chip.
- Ligand Capturing: Capture Human CD19, Fc Tag to the CM5 chip with immobilized Anti-human IgG (Fc) antibody.
- Analysis: A series of FMC63 mAb concentrations including 0nM, 1.25nM, 2.5nM, 5nM, 10nM, 20nM, and 40nM were injected consecutively, each with a contact time of 120 seconds and a dissociation time of 480 seconds.
- Regeneration: The chip was regenerated using 3 M magnesium chloride.

>>>Experimental Results





Product	Vendor	Method	Ligand	Capture level	Analyte Conc.	ka (1/Ms)	kd (1/s)	KD(M)	Rmax (RU)	% Bound
Human CD19, Fc Tag (Cat.No. CD9-H5251)	**ACRO**	Anti-human IgG capture	Fc-CD19	220 RU	FMC63 6-0.19ug/ml (40-1.25nM)	950378.1	0.00016	1.69E-10	50.84	17.6%
Human CD19, Fc Tag	**Another Vendor**		Fc-CD19	221 RU		815157.7	0.000281	**3.44E-10**	**19.20**	**6.6%**

>>>Conclusions

ACRO's rhCD19-Fc exhibited better performance in SPR assay compared to that of the counterpart from another vendor under the same conditions. The affinity constant (KD value) of ACRO's rhCD19-Fc to FMC63 was 0.17 nM, which was 2-fold higher than the counterpart from another vendor.

Reference

Nicholson, Ian C. et al. "Construction and characterisation of a functional CD19 specific single chain Fv fragment for immunotherapy of B lineage leukaemia and lymphoma." Molecular Immunology, vol. 34, No. 16-17, 1157-1165, 1997.