

CD47/SIRP α -related *in vitro* assay platform



WuXi AppTec, WuXi Biology, Oncology & Immunology Unit



2022.06

OncoWuXi Newsletter

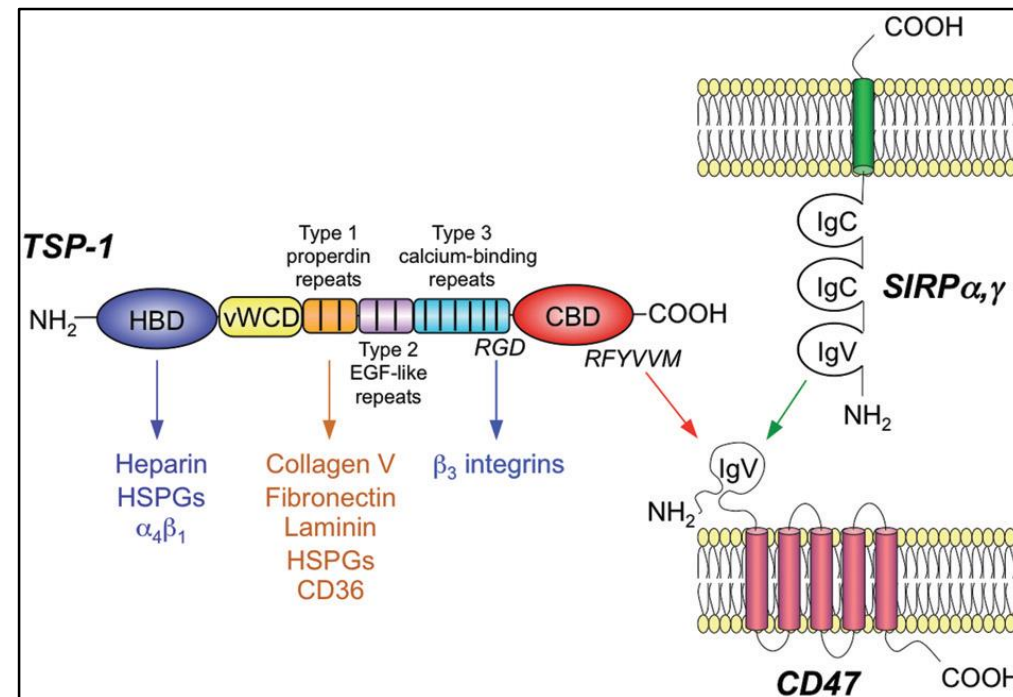
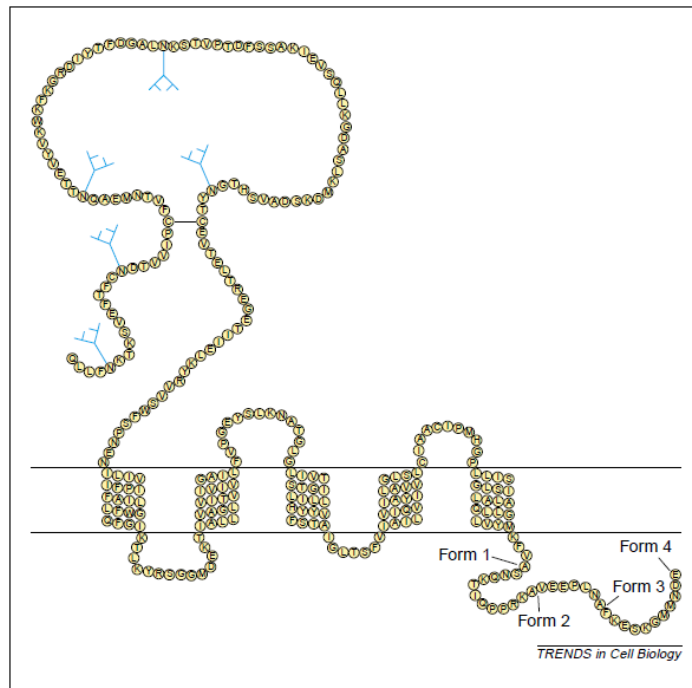
Outline

- CD47/SIRP α checkpoint biology
- *In vitro* functional assessments

CD47/SIRP α checkpoint biology

CD47 structures

- CD47 is a 32-35 kDa (45-55 kDa, glycosylated migration) with five transmembrane IgSF glycoprotein that broadly expressed on normal tissues and overexpressed on many tumors
- Alternatively spliced cytoplasmic tails give rise to four isoforms (I - IV)
 - II isoform (1st predominant): in hematopoietic, vascular endothelial, and epithelial cells
 - I isoform: in keratinocytes
 - III & IV (2nd abundant & longest) isoform: in neuron, intestinal mucosal cells and testicular cells
- As a signaling receptor for thrombospondin-1 (TSP1) and the counter-receptor for SIRP α endogenously



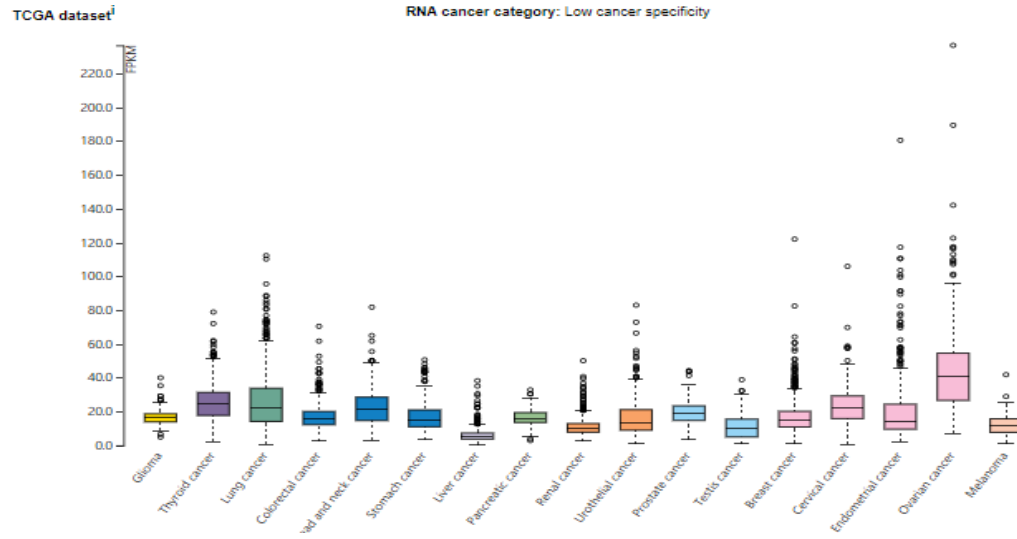
Interaction of CD47 and its endogenous ligands TSP-1 and SIRP α/γ

CD47/SIRP α checkpoint biology

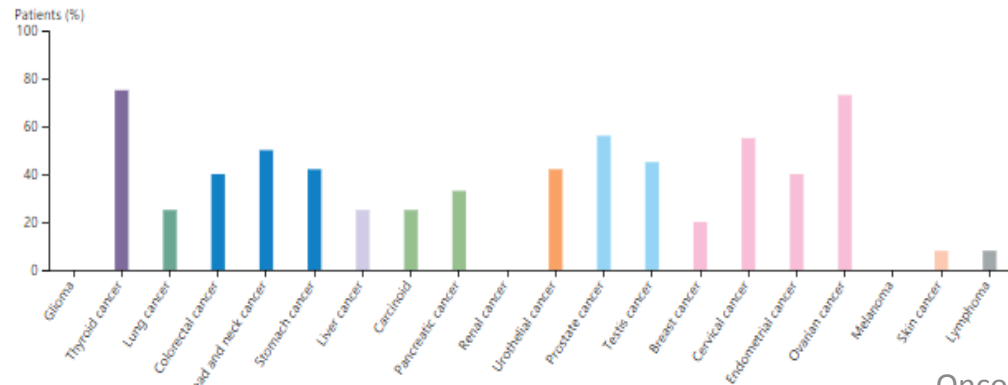
CD47 expression

- CD47 is ubiquitously expressed on normal tissues and broadly over-expressed in different types of tumors

- ✓ CD47 RNA cancer category



- ✓ CD47 is overexpressed in different types of tumors



- ✓ Copy number of CD47 in several tumor cell lines

Species	Cell type	Mean CD47 RN
Human Tumor Cell Lines	OV90	2,314,058
	HCC827	505,414
	SNU-1	389,435
	OV10-315	336,009
	MDA-MB-231	208,222
	Jurkat	233,026
Raji	117,640	

- ✓ Copy number of CD47 in normal cells

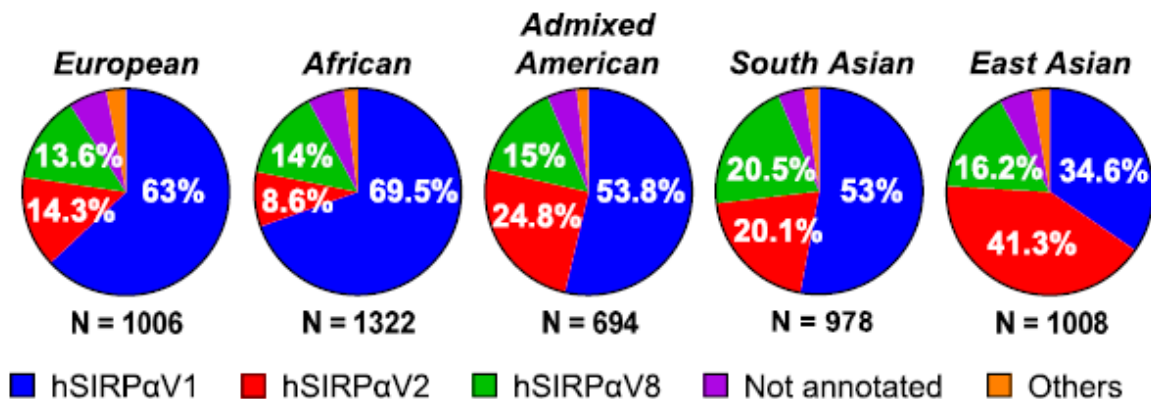
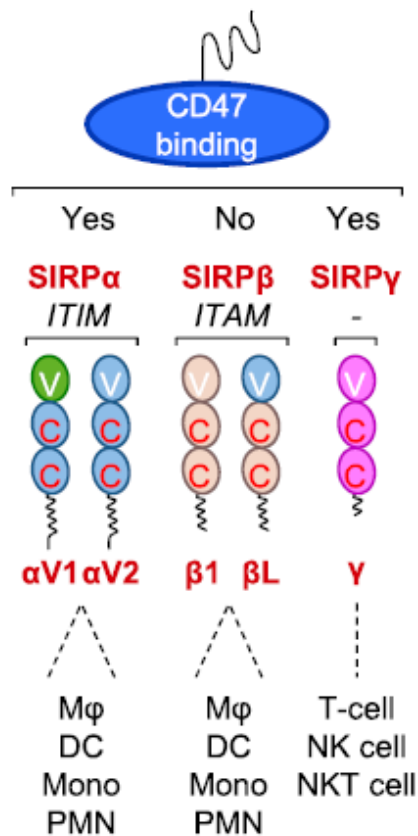
Species	Cell type	Mean CD47 RN
Human	RBCs	32,086
	Platelets	34,439
	Naïve CD3+ T cells	83,477
	Activated CD3+ T cells	N.D.
	Endothelial	48,944
	Epithelial	115,784
	Skeletal	85,661
Cynomolgus monkey	RBCs	26,087
	Platelets	34,728
	CD3 ⁺ T cells	35,800

*Mol Cancer Ther, 2020, 19(3), 835-47
Adapted from the human protein atlas*

CD47/SIRPα checkpoint biology

SIRPα structures & expression

- Signal regulatory protein a (SIRPα) is a transmembrane IgSF glycoprotein, with 3 Ig-like ECDs, a TMD and a CD contains ITIM
- SIRPα, SIRPβ and SIRPγ belong to the class of paired receptors comprising separate genes that encode proteins with similar ECD but different TM or CD



C

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hSIRPαV1  GVAGEEELQVIQPKSVLVAAGETATLRCTATSLIPVGP IQWFRGAGPGRELIYNQKEGH
hSIRPαV2  GVAGEEELQVIQPKSVLVAAGESALILCTVTSIPVGP IQWFRGAGPARELIYNQKEGH
hSIRPαV8  GVAGEEELQVIQPKSVLVAAGETATLRCTATSLIPVGP IQWFRGAGPARELIYNQKEGH
*****:*****:*:*:*****
hSIRPαV1  FPRVTTVSDLTKRNNMDFSI RIGNITPADAGTY YCVKFRKGS PDDVEFKSGAGTELSVRA
hSIRPαV2  FPRVTTVSESTKRENMDFSI RIGNITPADAGTY YCVKFRKGS PDDVEFKSGAGTELSVRA
hSIRPαV8  FPRVTTVSESTKRENMDFSI RIGNITPADAGTY YCVKFRKGS PDDVEFKSGAGTELSVRA
*****:***:*****:*:*:*****
    
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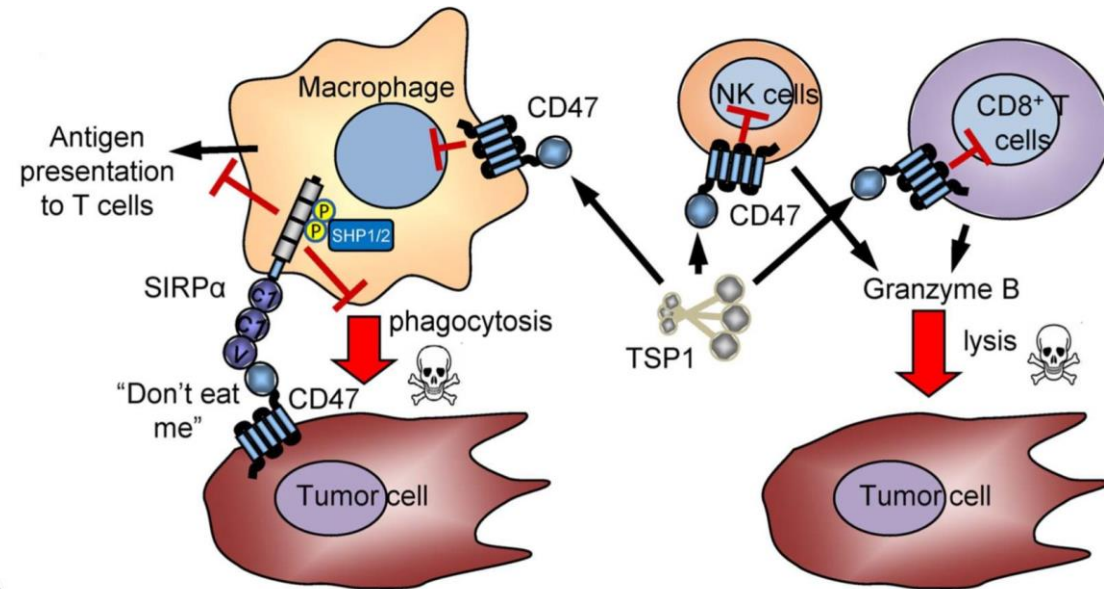
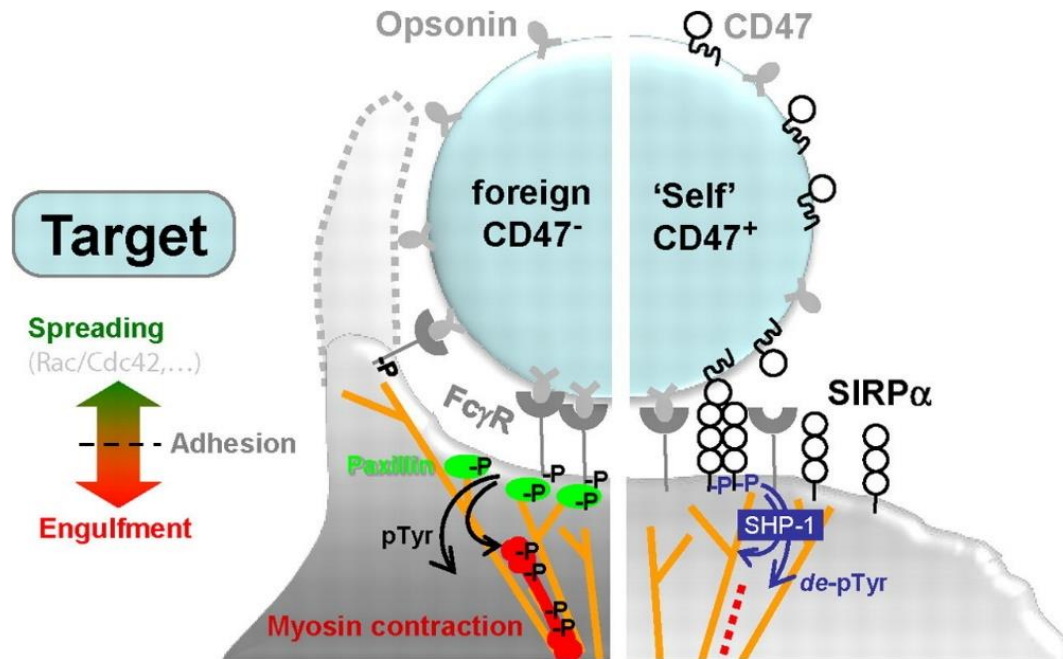
IgV domain

JITC, 2019, 7, 340

CD47/SIRP α checkpoint biology

Pathophysiological functions

- CD47 delivers an inhibitory “don’t eat me” signal to macrophages through SIRP α



- ✓ The expression of CD47 is used by macrophages to distinguish between “self” or “non-self”

- ✓ Tumors use CD47 “don’t eat me” signal to evade destruction by innate immune system

Antib Ther, 2020, JNL, 1-14
PNAS, 2012, 109(17):6662-7

MOA of CD47/SIRP α checkpoint axis blockade

- Macrophage mediated phagocytosis
 - CD47 mAbs, SIRP α mAbs, or SIRP α fusion protein
- Promoting phagocytic uptake & antigen presentation
 - CD47 mAbs
- ADCC & CDC
 - CD47 mAbs
- Caspase-independent apoptosis
 - CD47 mAbs

Active clinical trials targeting CD47

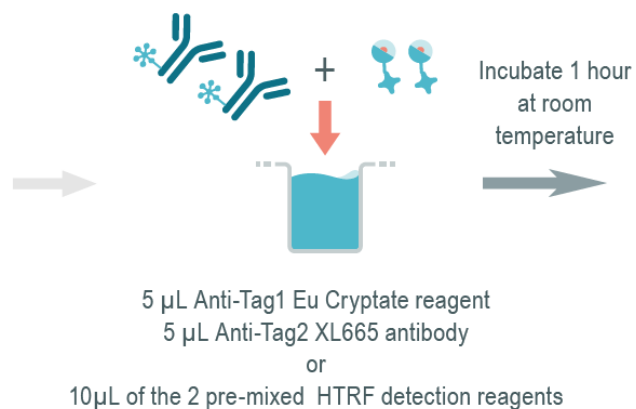
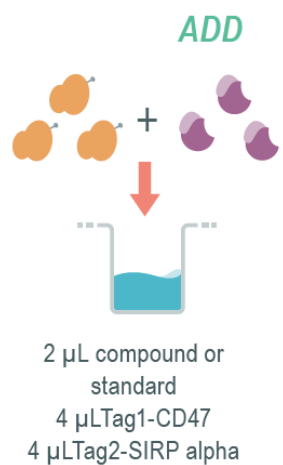
Molecule ID	Molecule type	Company	Phase	Tumor type	Combination
RRx-001	Small molecule	EpicentRx	III	SCLC & neuroendocrine tumors & ovarian cancer	N/A
Magrolimab	CD47 mAb, IgG4, 150 kDa	Gilead & Forty Seven	III	Solid tumors & NHL & B cell lymphoma	Single agent or +Cetuximab & Prism Study & +Rituximab, Azacitidine
TTI-621	SIRP α -IgG1 Fc fusion protein, 75 kDa	Trillium	II	Solid & hematologic cancers & mycosis fungoides	+Rituximab, Nivolumab & +PD-1/PD-L1, PEG-IFN- α 2a, T-Vec, radiation
TTI-622	SIRP α -IgG4 Fc fusion protein, 75 kDa	Trillium	II	Lymphoma & myeloma	+Rituximab, proteasome-inhibitor, PD-1/PD-L1
AO-176	CD47 mAb, IgG2, 150 kDa	Arch Oncology	I/II	Solid tumors	Single agent
IBI188	CD47 mAb, IgG4, 150 kDa	Innovent	I	Advanced malignancies	Single agent & +Rituximab
IBI322	PD-L1/CD47 bsAb, 125 kDa	Innovent	I	Advanced tumors	+PD-1/PD-L1
Lemzoparlimab	CD47 mAb, IgG4, 150 kDa	Abbvie, I-Mab Biopharma	I	Solid tumors & NHL	Single agent
ALX148	SIRP α -IgG1 Fc fusion protein	Alexo Therapeutics	I	Solid tumors & lymphoma	+Azacitidine
TG-1801	CD47/CD19 bsAb	TG Therapeutics	I	B cell lymphoma	+Pembrolizumab, Trastuzumab, Rituximab
SRF231	CD47 mAb, IgG4	Surface Oncology	I	Solid tumors & hematologic cancers	Single agent
SGN CD47M	ADC	Seattle Genetics	I	Solid tumors	Single agent
HX-009	PD-1/CD47 bsAb	Waterstone Hanxbio	I	Solid tumors	Single agent
IMC-002	CD47 mAb	ImmuneOncia	I	Solid tumors and lymphoma	Single agent
CC-9002	CD47 mAb, IgG4, 150 kDa	Celgene, Inhibrx	I	Solid & hematologic cancers	+Rituximab

In vitro functional assessments targeting CD47/SIRP α interaction

- CD47-SIRP α interaction blocking/inhibitory assay (for screening)
 - *HTRF & AlphaLISA blocking/inhibitory assays (Protein based)*
 - *Blocking/inhibitory assay (Cell based)*
- *In vitro* effector function
 - *FACS & cell based binding assays*
 - *Phagocytosis assay*
- Potential safety assessments
 - *Antibody binding assay on RBCs*
 - *Hemolysis assay*

CD47-SIRP α interaction blocking/inhibitory assay

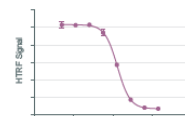
Protein based HTRF blocking assays



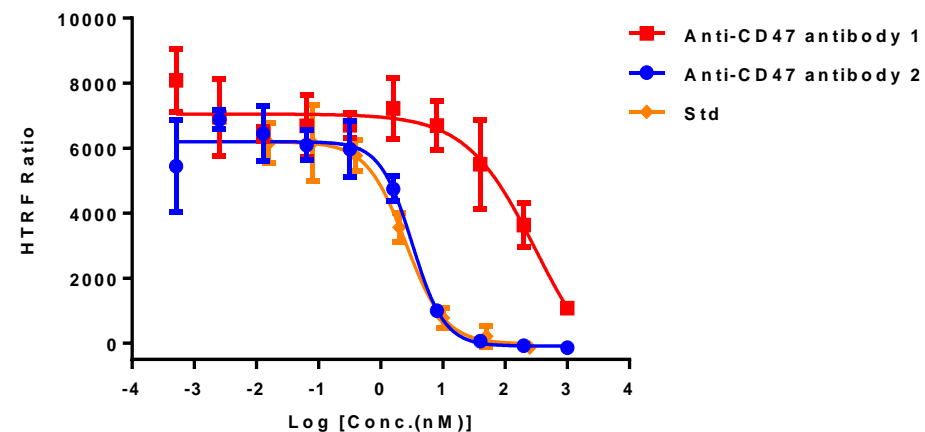
READ



ANALYSE



HTRF CD47/SIRP α Blocking Assay



	Anti-CD47 antibody 2	Anti-CD47 antibody 1	Std
IC50	3.216	313.3	2.522

<https://www.perkinelmer.com.cn>

CD47-SIRP α interaction blocking/inhibitory assay

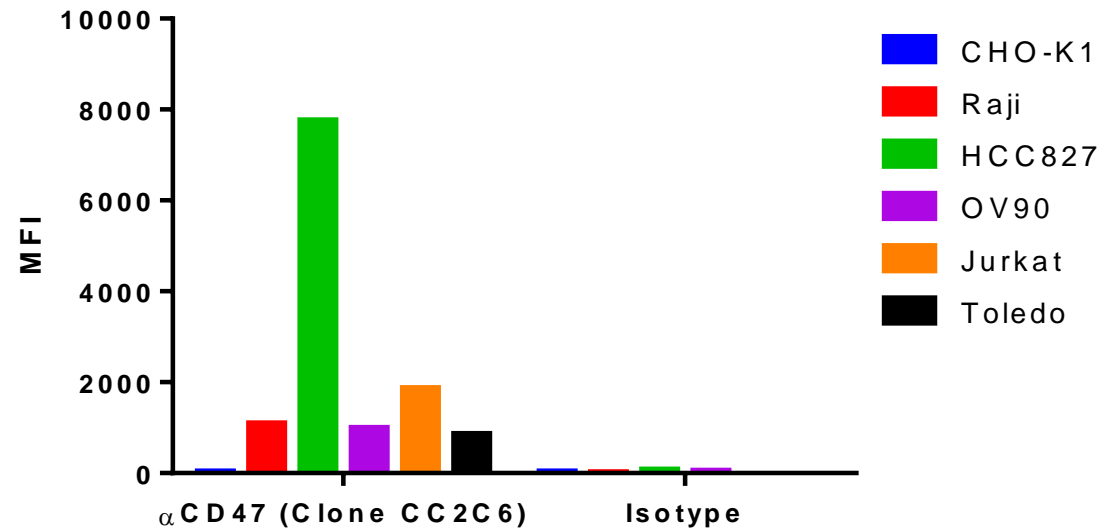
CD47 expression validation by CD47 antibody

Plate the cells with 200,000 cells/well in 50 μ L of growth medium at a round-bottom 96-well plate

Add 50 μ L of anti-CD47 antibody (Clone CC2C6) with the recommend conc. of and incubate for 30-40 min at 4 $^{\circ}$ C

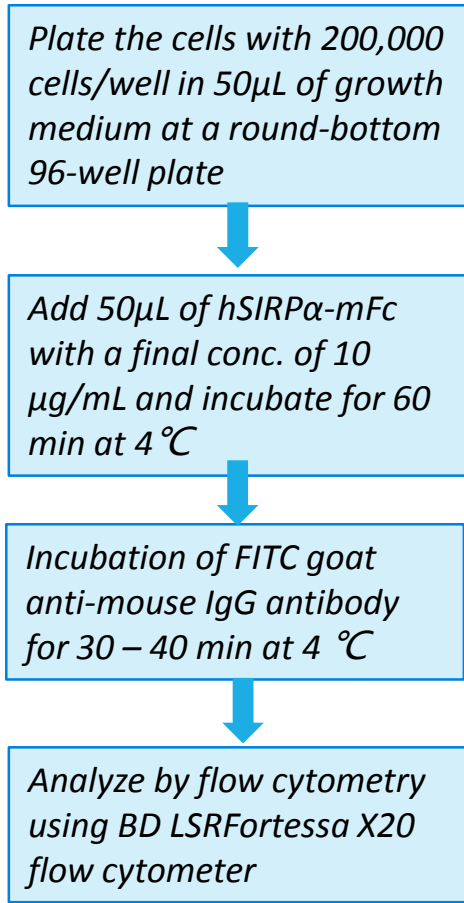
Analyze by flow cytometry using BD LSRFortessa X20 flow cytometer

Cell CD47 expressing level detection using anti-CD47 (Clone CC2C6)

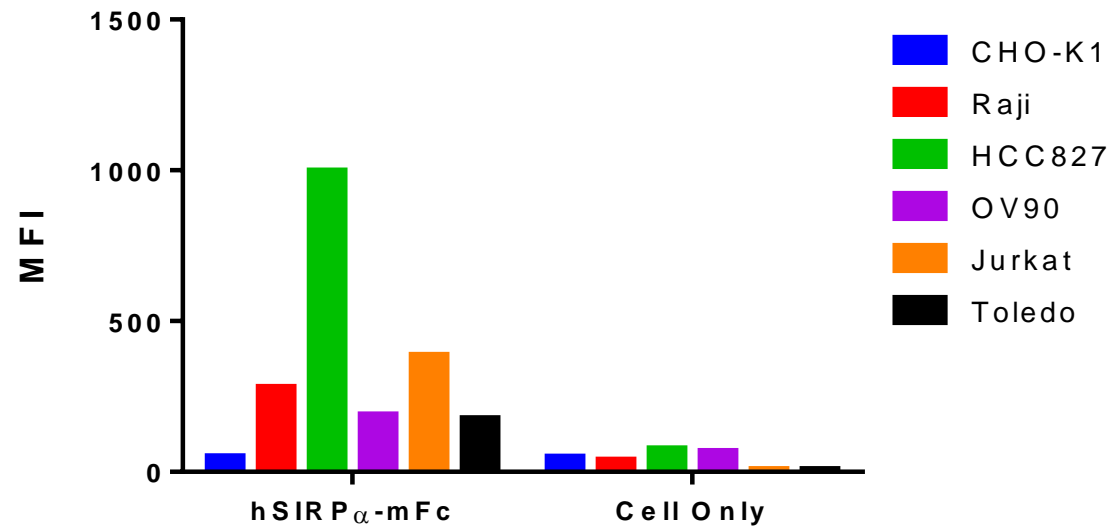


CD47-SIRP α interaction blocking/inhibitory assay

CD47 expression validation by hSIRP α -mFc

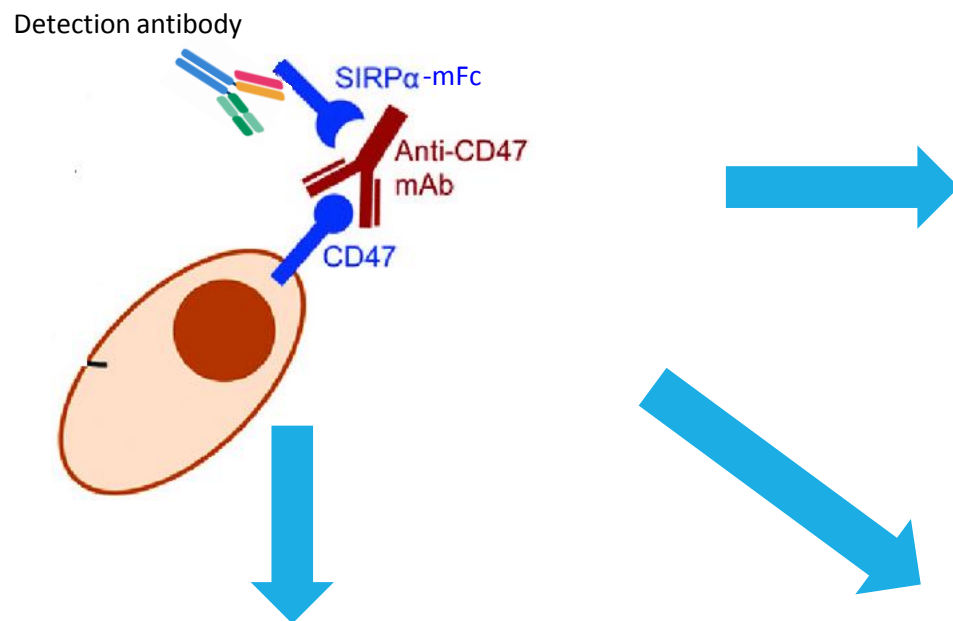


Cell CD47 expressing level detection using hSIRP α -mFc

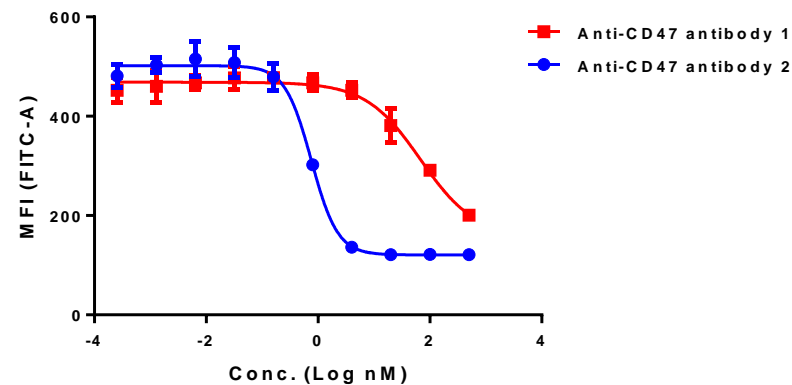


CD47-SIRP α interaction blocking/inhibitory assay

Cell based blocking/inhibitory assay

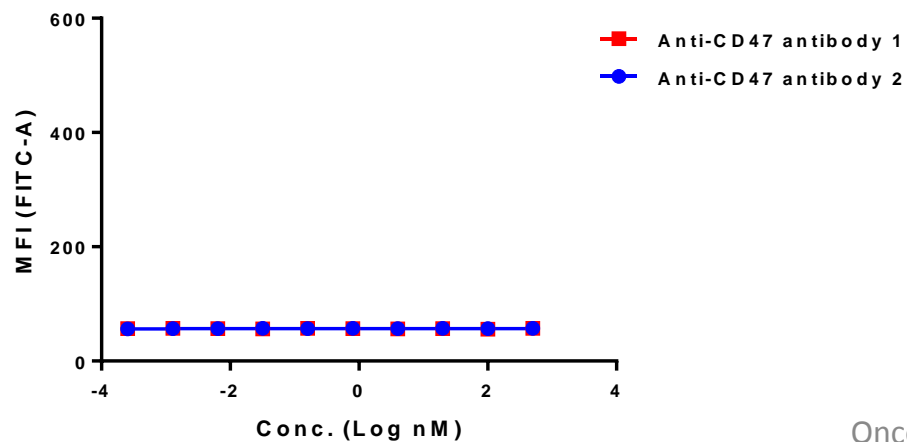


Cell based blocking of CD47/SIRP α interaction (HCC827^{High})

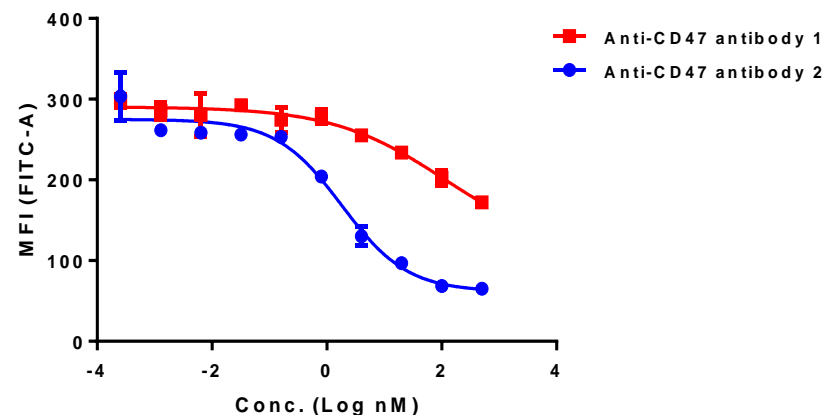


	Anti-CD47 antibody 2	Anti-CD47 antibody 1
IC50	0.7574	67.58

Cell based blocking of CD47/SIRP α interaction (CHO-K1^{neg})



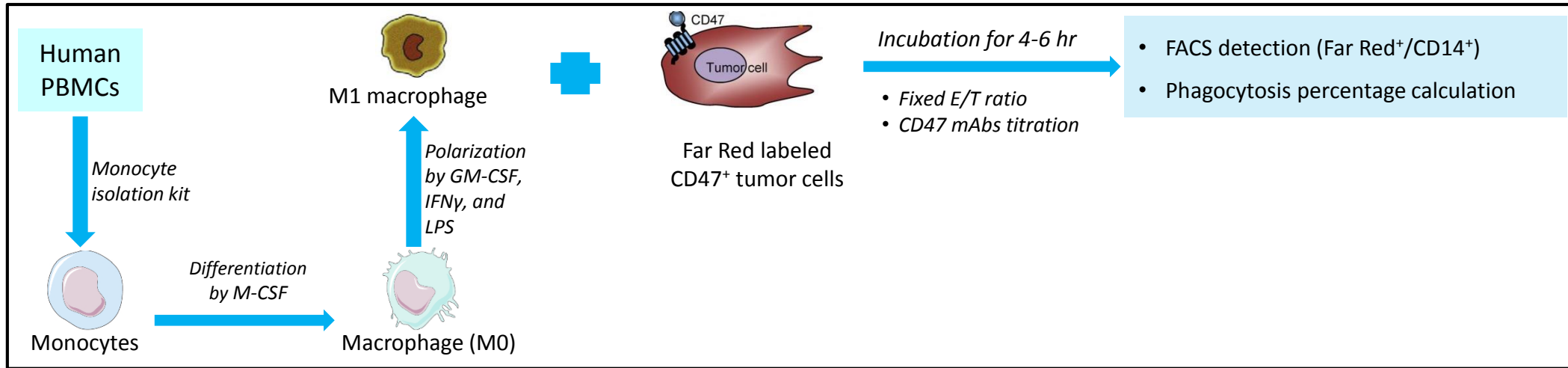
Cell based blocking of CD47/SIRP α interaction (Raji^{low})



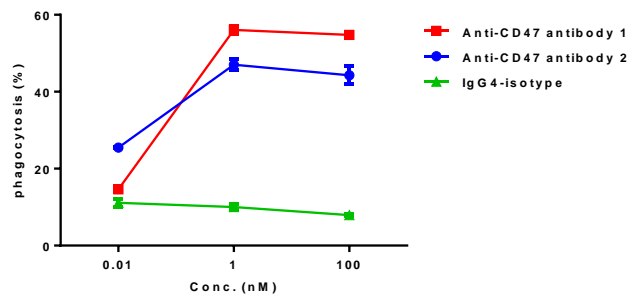
	Anti-CD47 antibody 2	Anti-CD47 antibody 1
IC50	1.820	109.6

CD47 mAb mediated phagocytosis (ADCP assay)

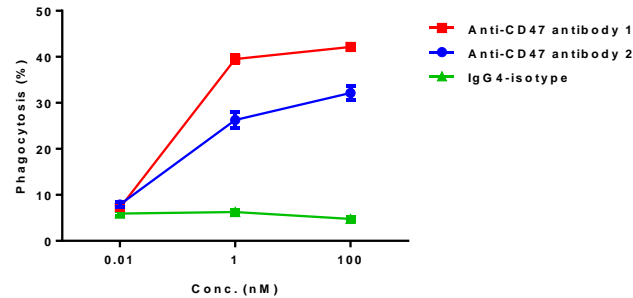
M1 mediated phagocytosis



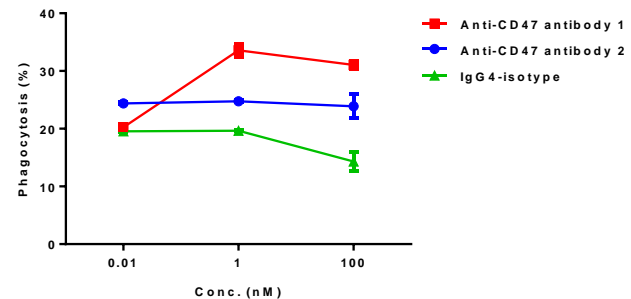
CD47 mAb mediated ADCP to Toledo cells (CD47^{High}, E/T=5)



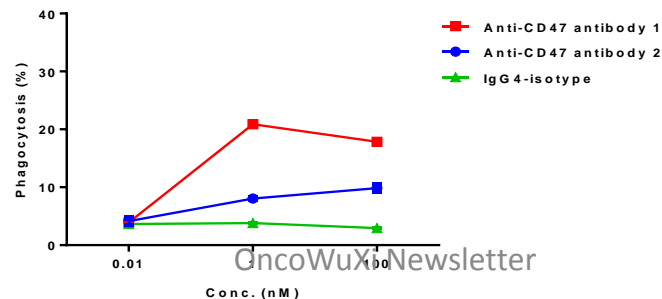
CD47 mAb mediated ADCP to Jurkat cells (CD47^{Med}, E/T=5)



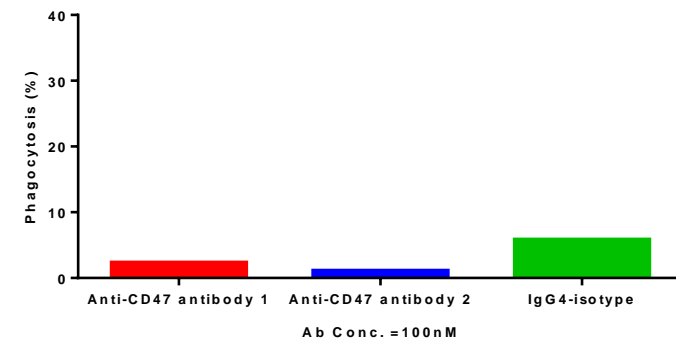
CD47 mAb mediated ADCP to NCI-H82 cells (CD47^{High}, E/T=5)



CD47 mAb mediated ADCP to Raji cells (CD47^{Low}, E/T=5)

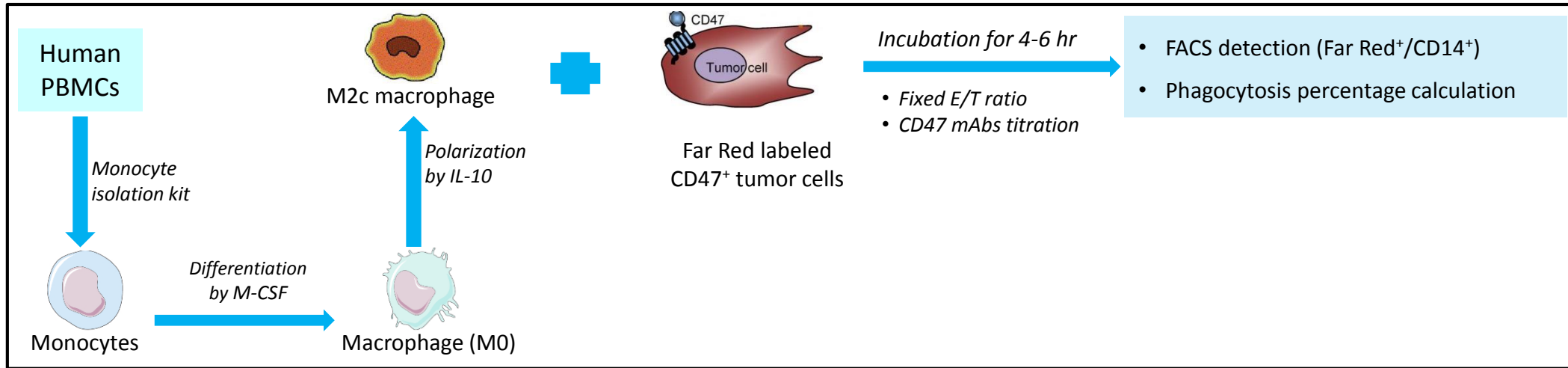


CD47 mAb mediated ADCP to CHO-K1 cells (CD47^{Neg}, E/T=5)

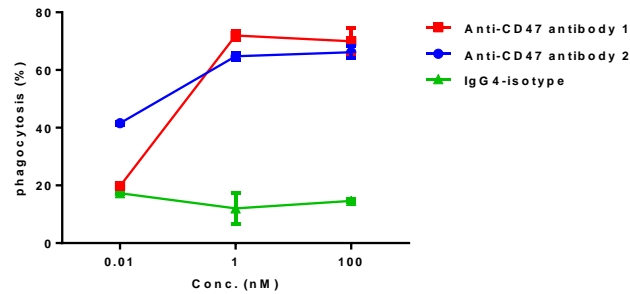


CD47 mAb mediated phagocytosis (ADCP assay)

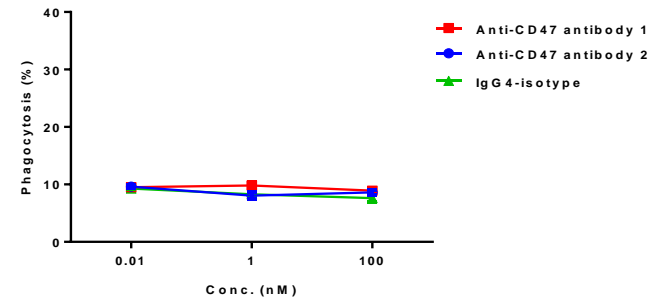
M2c mediated phagocytosis



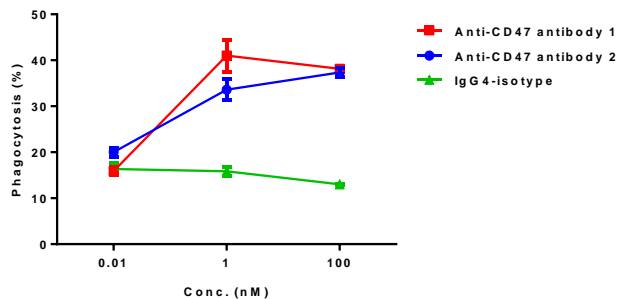
CD47 mAb mediated ADCP to Toledo cells (CD47^{High}, E/T=5)



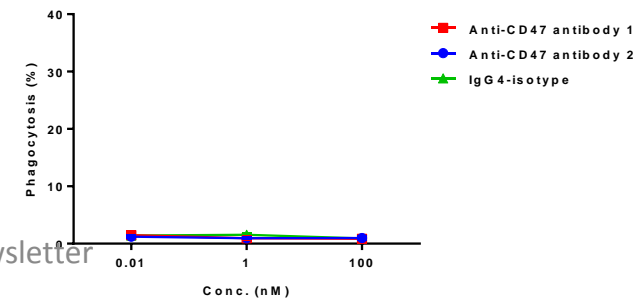
CD47 mAb mediated ADCP to Raji cells (CD47^{Low}, E/T=5)



CD47 mAb mediated ADCP to Jurkat cells (CD47^{Med}, E/T=5)



CD47 mAb mediated ADCP to CHO-K1 cells (CD47^{Neg}, E/T=5)



Potential safety assessments

CD47 binding assay by CD47 antibody on RBC of cynomolgus monkey

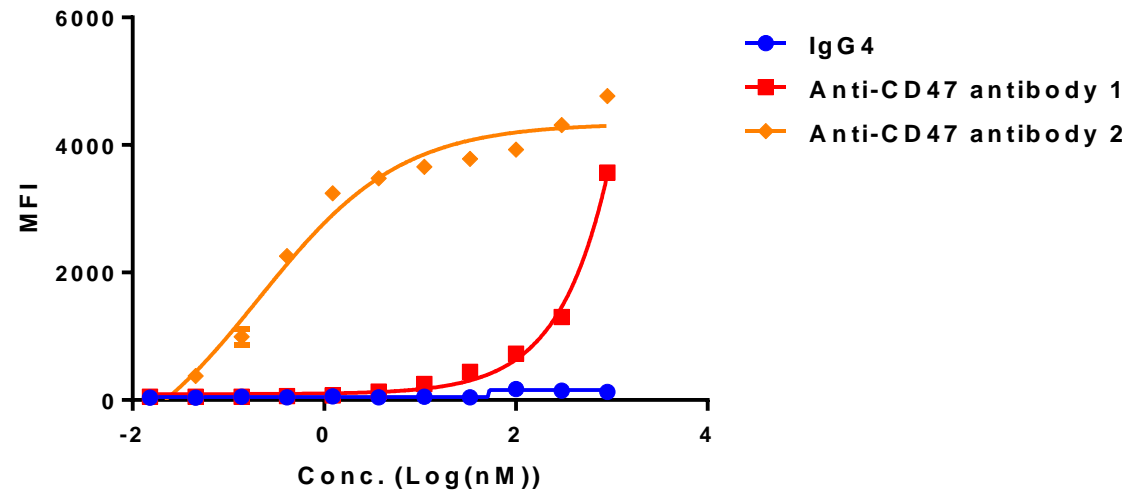
- To validate the CD47 binding capability on RBCs of cynomolgus monkey

Plate the cells with 500,000 cells/well in 50 μ L of growth medium at a round-bottom 96-well plate

Add 50 μ L of anti-CD47 antibodies with the series of concentrations and incubate for 30-40 min at 4 $^{\circ}$ C; then wash cells, add 50 μ L secondary fluorescence antibody and incubate for 30-40 min at 4 $^{\circ}$ C.

Analyze by flow cytometry using BD LSRFortessa X20 flow cytometer

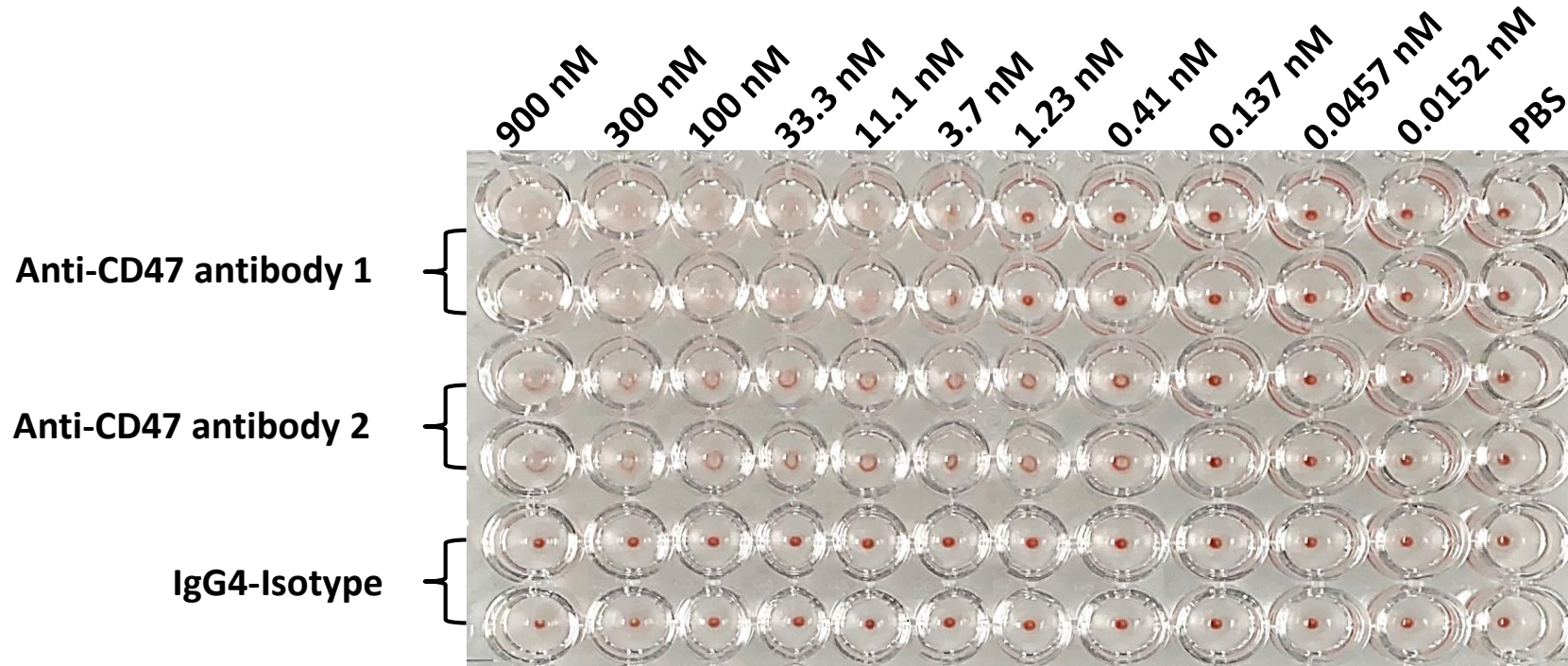
CD47 mAb binding assay on RBCs of cynomolgus monkey



Potential safety assessments

Hemolysis assay

- To validate the Hemolysis on RBCs of cynomolgus monkey





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