



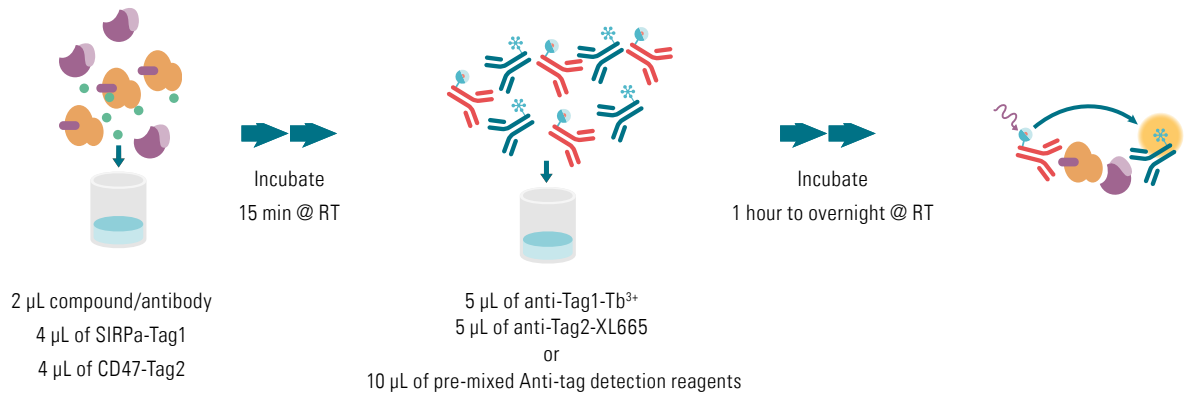
CD47/SIRPa BINDING ASSAY

TECHNICAL NOTE

ABSTRACT Signal-regulatory protein a (SIRP-a), also known as CD172a, is a cell surface protein expressed mainly by myeloid cells such as macrophages. Its receptor, CD47, is ubiquitously expressed on the surface of all cells and has been found to be overexpressed in some cancers. CD47 binding to SIRP-a delivers a “Do Not Eat Me” signal to macrophages. In the context of a tumor, this signaling aids in tumor evasion of the immune system. Inhibitors of this interaction hold great promise for cancer immunotherapy.

The HTRF CD47/SIRPa Binding Assay is designed to measure the interaction between CD47 and SIRPa proteins. Utilizing HTRF (Homogeneous Time-resolved Fluorescence) technology, the assay enables simple and rapid characterization of compound and antibody (including human antibody) blockers in a high throughput format.

INHIBITION TEST



Reagents should be dispensed in the following order:

- 2 µL compound/antibody or diluent buffer.
- 4 µL SIRPa-Tag1.
- 4 µL CD47-Tag2.

Incubate at RT for 15min.

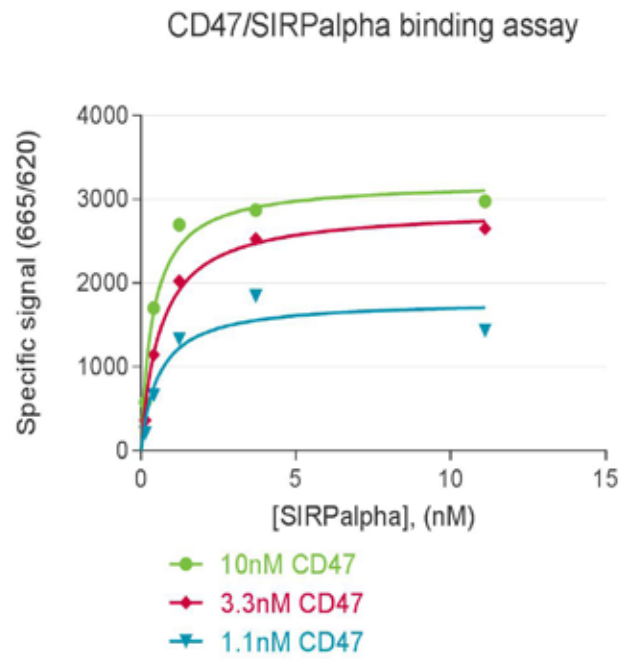
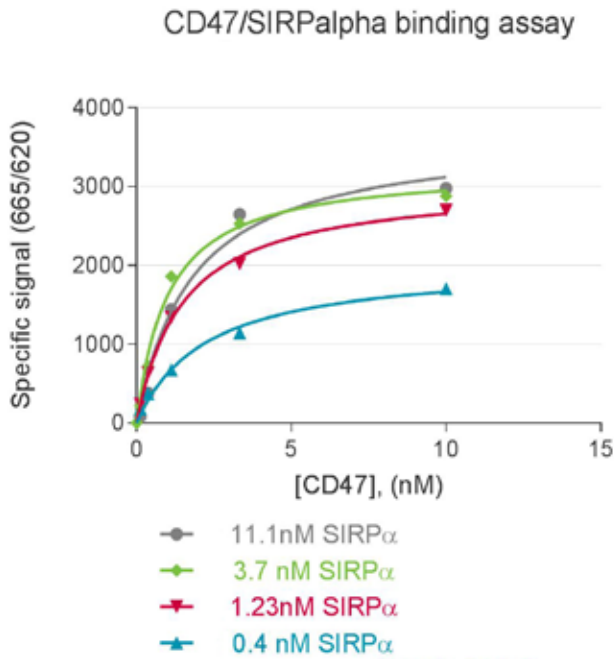
- 5 µL of anti-Tag1-Tb³⁺ and 5 µL of anti-Tag2-XL665 or 10 µL of pre-mixture of two conjugates.

Seal the plate and incubate at RT for 1 hour to overnight.

Remove the plate sealer and read the fluorescence emission.

	Inhibitor	Tag1-SIRPa	Tag2-CD47	Anti-Tag1-Cryptate	Anti-Tag2-XL665	Diluent	Detection buffer
Sample	2 µL	4 µL	4 µL	5 µL	5 µL		
Positive control		4 µL	4 µL	5 µL	5 µL	2 µL	
Negative control			4 µL	5 µL	5 µL	6 µL	
Cryptate control				5 µL		10 µL	5 µL
Buffer control						10 µL	10 µL

For 96 & 384-well low volume plates (20 µL).



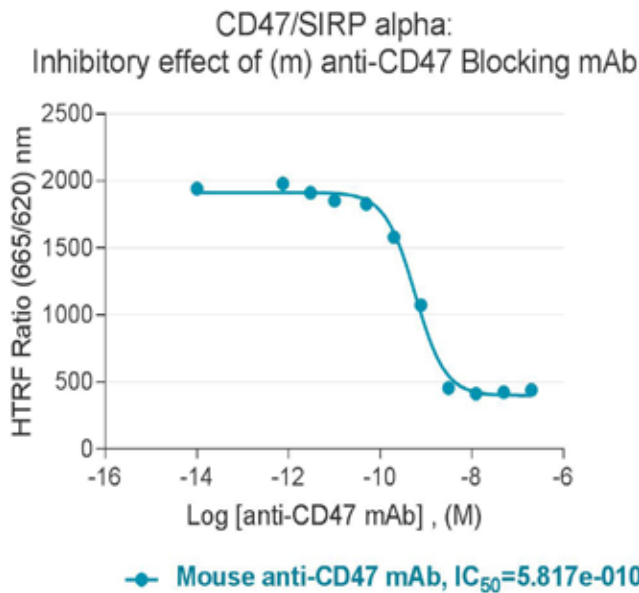
In order to optimize protein concentrations, the 2 tagged proteins were cross-titrated. CD47 was titrated from 0 to 10 nM at 0.4, 1.23, 3.7 and 11.1 nM of SIRPα and SIRPα titration, ranging from 0 to 10 nM, was achieved at 1.1, 3.3 and 10 nM of CD47. The two proteins were incubated for 1 hour at room temperature.

Data were calculated by fitting specific signal to One-site specific binding with hill slope.

Specific signal for each ligand concentration = Total binding - Non specific binding

2 nM CD47 and 1 nM SIRPα were chosen for inhibition tests.

INHIBITION OF CD47/SIRPA



The inhibitory effects of Mouse anti-CD47 were tested at 2nM CD47 and 1 nM SIRPα. An assay window around 4 was obtained.