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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

TIGIT:CD112 Homogeneous Assay Kit

Catalog #72030
Size: 384 reactions

BACKGROUND: Human T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is a receptor that is expressed on the surface of human T cells and NK cells that binds to CD155 and CD112 on the surface of dendritic cells. Binding of TIGIT with CD155 or CD112 results in inhibition of T cell and NK cell activation. Antibodies and other agents that inhibit this signaling pathway have been shown to increase the immune response, especially in the case of certain cancers.

DESCRIPTION: The *TIGIT:CD112 Homogeneous Assay Kit* is designed to measure the inhibition of human T-cell immunoreceptor with Ig and ITIM domains (TIGIT) binding to CD112 (PVRL2/Nectin-2). The *TIGIT:CD112 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format with purified biotinylated TIGIT, His-tagged CD112, and assay buffer to perform a total of 384 reactions. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing TIGIT and an inhibitor of choice is incubated with the CD112 for 60 minutes. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71251	TIGIT-Fc-biotin	2x 5 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71197	CD112-His	2x 6 µg	-80°C	
79311	3x Immuno Buffer 1	4 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA Ni Chelate Acceptor beads, 5 mg/ml (PerkinElmer #AL108C)
AlphaScreen Streptavidin-conjugated Donor beads, 5 mg/ml (PerkinElmer #6760002S)
Optiplate-384 (PerkinElmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for screening for inhibitors of TIGIT binding to CD112

CONTRAINDICATIONS: Only limited amounts of DMSO can be included, as it has been shown to disrupt TIGIT:CD112 interaction. Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due

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to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

STABILITY: At least one year from date of receipt when stored as directed.

REFERENCES: 1. Yu, X., *et al., Nat. Immunol*. 2009; **10(1):** 48-57.
2. Stanietsky, N., *et al., Proc. Natl. Acad. Sci.* 2009; **106(42):** 17858-17863.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

Step 1:

1) Thaw **CD112-His** on ice. Upon first thaw, briefly spin tube containing protein to recover full contents of the tube. Aliquot the protein into single use aliquots. Store remaining undiluted protein in aliquots at -80°C immediately. *Note: **CD112-His** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein*.

2) Dilute one part **3x Immuno Buffer 1** with 2 parts of distilled water (3-fold dilution) to make **1x Immuno Buffer 1**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

3) Dilute **CD112-His** in **1x Immuno Buffer 1** to 5 ng/µl. Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use.

4) Prepare the master mixture: N wells × (2 µl **3x Immuno Buffer 1** + 2 µl diluted **CD112-His** + 2 µl distilled water). Add 6 µl of master mixture to every well.

	Blank	Positive Control	Test Inhibitor
3x Immuno Buffer 1	2 µl	2 µl	2 µl
CD112-His (5 ng/µl)	2 µl	2 µl	2 µl
Distilled water	2 µl	2 µl	2 µl
Test Inhibitor	–	–	2 µl
Inhibitor buffer (no inhibitor)	2 µl	2 µl	–
1x Immuno Buffer 1	2 µl		
TIGIT-biotin (4 ng/µl)	–	2 µl	2 µl
Total	**10 µl**	**10 µl**	**10 µl**

5) Add 2 µl of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control” and “Blank”, add 2 µl of the same solution without inhibitor (inhibitor buffer). *Note: If possible, keep final DMSO concentration below 0.5%.*

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6) Add 2 µl of **1x Immuno Buffer 1** to the well designated "Blank".

7) Thaw **TIGIT-biotin** on ice. Upon first thaw, briefly spin tube containing protein to recover full contents of the tube. Aliquot the protein into single use aliquots. Store remaining undiluted protein in aliquots at -80°C immediately. *Note: **TIGIT-biotin** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

8) Dilute **TIGIT-biotin** in **1x Immuno Buffer 1** to 4 ng/µl. Keep diluted proteins on ice until use. Discard any remaining unused diluted protein after use.

9) Initiate reaction by adding 2 µl of diluted **TIGIT-biotin** prepared as described above to each well designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for 60 minutes.

Step 2:
Note: Protect your samples from direct exposure to light!

1) Dilute Ni Chelate Acceptor beads (PerkinElmer #AL108C) 250-fold with **1x Immuno Buffer 1**. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:
Note: Protect your samples from direct exposure to light!

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x Immuno Buffer 1**. Add 10 µl per well. Incubate at room temperature for 30 minutes.

2) Read Alpha-counts.

Due to lot to lot variability in AlphaScreen® *bead performance, it may be necessary to optimize assay conditions. For example, slight adjustments to TIGIT-biotin or CD112-His concentrations may improve signal-to-noise ratio.*

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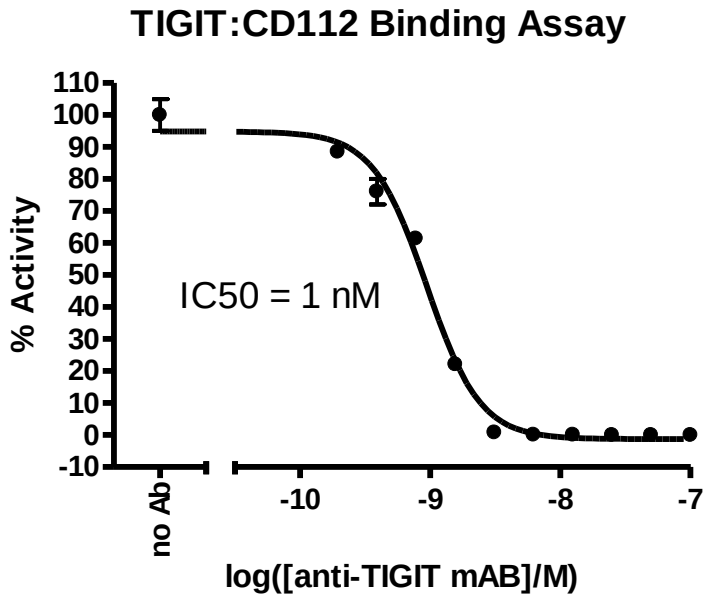
Example of Assay Results:



TIGIT:CD112 inhibition, measured using the TIGIT:CD112 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72030 and an anti-TIGIT antibody (BPS Cat. #71218). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

Product Name	Catalog #	Size
Human TIGIT	#71218	100 µg
Human TIGIT, Fc fusion, Biotin-labeled	#71251	50 µg
Human TIGIT, Fc fusion	#71186	100 µg
Human CD112, His-tag	#71197	100 µg
Human CD112, His-tag, Biotin-labeled	#71234	50 µg
Human CD155 (PVR), His-tag	#71181	100 µg
Mouse CD155 (PVR), His-tag	#71167	100 µg
Mouse CD155 (PVR), His-tag, Biotin-labeled	#71168	50 µg
TIGIT:CD155 Homogeneous Assay Kit	#72029	384 rxns.