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Data Sheet
CD226:CD112 Homogeneous Assay Kit
Catalog #72051
Size: 384 reactions

BACKGROUND: CD226 is an activating receptor expressed on the surface of natural killer (NK) cells, monocytes, platelets, and T cells. Its natural ligands are CD112 and CD155 which are expressed by antigen presenting cells (APC). Binding of CD226 to either CD112 or CD155 triggers NK cell effector function and can promote Th1 cell differentiation in T cells. CD226 competes for binding with CD112 and CD155 with the co-inhibitory receptor TIGIT. Agonistic CD226 monoclonal antibodies are being explored as potential therapeutics in settings where immune activation is beneficial, such as cancer.

DESCRIPTION: The *CD226:CD112 Homogeneous Assay Kit* is designed to measure the inhibition of CD226 binding to CD112 (PVRL2). The *CD226:CD112 Homogeneous Assay Kit* comes in a convenient AlphaLISA[®] format with purified biotinylated CD226, 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 CD226 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	
71253	CD226-Fc-biotin	2x 8 µg	-80°C	<i>(Avoid freeze/ thaw cycles!)</i>
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 CD226 binding to CD112

CONTRAINDICATIONS: Only limited amounts of DMSO can be included, as it has been shown to disrupt CD226: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 (NaN₃) or metal ions (Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Ni²⁺). 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. Bottino, C. *et al.*, *J. Exp. Med.* 2003; **198(4)**: 557-567.
2. Pende, D. *et al.*, *Blood.* 2005; **105(5)**: 2066-2073.

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		
CD226-biotin (6 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 μ l of **1x Immuno Buffer 1** to the well designated "Blank".
- 7) Thaw **CD226-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: **CD226-biotin** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
- 8) Dilute **CD226-biotin** in **1x Immuno Buffer 1** to 6 ng/ μ l. Keep diluted proteins on ice until use. Discard any remaining unused diluted protein after use.
- 9) Initiate reaction by adding 2 μ l of diluted **CD226-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 μ l per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

Note: Protect your samples from direct exposure to light!

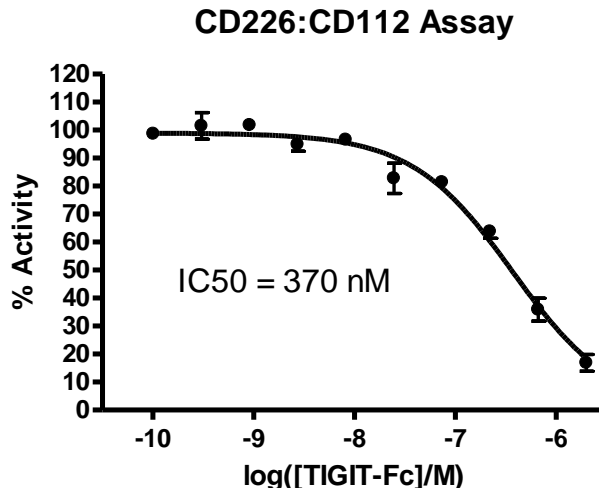
- 1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x Immuno Buffer 1**. Add 10 μ l per well. Incubate at room temperature for 30 minutes.
- 2) Read Alpha-counts.

Due to lot to lot variability in AlphaScreen[®] bead performance, it may be necessary to optimize assay conditions. For example, slight adjustments to CD226-biotin or CD112-His concentrations may improve signal-to-noise ratio.

Example of Assay Results:

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CD226:CD112 inhibition, measured using the CD226:CD112 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72051 and competing receptor TIGIT, Catalog #71186. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
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:CD112 Homogeneous Assay Kit	#72030	384 rxns.
TIGIT:CD155 Homogeneous Assay Kit	#72029	384 rxns.
CD226:CD155 Homogeneous Assay Kit	#72052	384 rxns.

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