Data Sheet

TIGIT:CD155 Homogeneous Assay Kit

Catalog #AMS.72029
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:CD155 Homogeneous Assay Kit* is designed to measure the inhibition of TIGIT binding to CD155. The *TIGIT:CD155 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format with purified biotinylated TIGIT, His-tagged CD155, 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 CD155 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	3 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71181	CD155-His	3 µg	-80°C	
	3x TIGIT assay buffer	4 ml	-20°C	

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

APPLICATIONS: Useful for screening for inhibitors of TIGIT binding to CD155

CONTRAINDICATIONS: Only limited amounts of DMSO can be included, as it has been shown to disrupt TIGIT:CD155 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 to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

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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 **CD155-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: **CD155-His** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein*.

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

3) Dilute **CD155-His** in **1x TIGIT assay buffer** to 2 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 TIGIT assay buffer** + 2 µl diluted **CD155-His** + 2 µl distilled water). Add 6 µl of master mixture to every well.

	Blank	Positive Control	Test Inhibitor
3x TIGIT assay buffer	2 µl	2 µl	2 µl
CD155-His (2 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 TIGIT assay buffer	2 µl		
TIGIT-biotin (2 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%.*

6) Add 2 µl of **1x TIGIT assay buffer** to the well designated “Blank”.

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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 TIGIT assay buffer** to 2 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 TIGIT assay buffer**. 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 TIGIT assay buffer**. 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 CD155-His concentrations may improve signal-to-noise ratio.*

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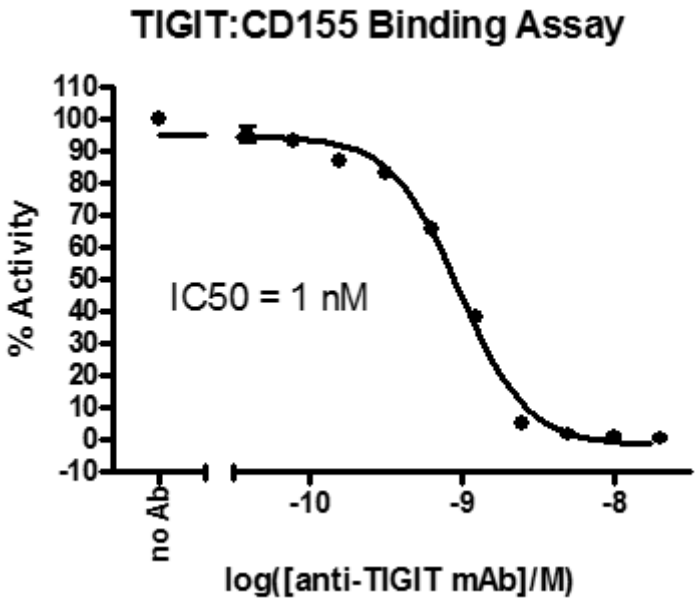
Example of Assay Results:



TIGIT:CD155 inhibition, measured using the TIGIT:CD155 Inhibitor Screening Assay Kit , Catalog #72029 and an anti-TIGIT antibody (AMSBIO Cat. #71218) *Data shown is lot-specific.*