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# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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**Description**

The Mouse TIGIT: Mouse CD155 (mTIGIT:mCD155) Homogeneous Assay Kit is designed to measure the inhibition of mTIGIT binding to mCD155. The mTIGIT:mCD155 Homogeneous Assay Kit comes in a convenient AlphaLISA® format with purified biotinylated Mouse TIGIT, His-tagged Mouse 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 mTIGIT and an inhibitor of choice is incubated with the mCD155 for 60 minutes. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

**Background**

T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is a receptor that is expressed on the surface of 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.

**Applications**

Useful for screening for inhibitors of TIGIT binding to CD155.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 79269 | Mouse TIGIT-Fc-biotin | 15 µg | -80°C |
| 71167 | Mouse CD155-His | 30 µg | -80°C |
| 79311 | 3x Immuno Buffer 1 | 4 ml | -20°C |

**Materials Required but Not Supplied**

| Name | Catalog # |
|---|---|
| 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 | |

**Storage Conditions**

This assay kit will perform optimally for up to one year from date of receipt when the materials are stored as directed.

**Safety**

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

**Contraindications**

Only limited amounts of DMSO can be included, as it has been shown to disrupt mTIGIT:mCD155 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 to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

**Assay Protocol**

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

*Preparing Your Reagents*

1. 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.
2. Thaw mTIGIT-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: mTIGIT-biotin is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.
3. Dilute mTIGIT-biotin in 1x Immuno Buffer 1 to 16 ng/µl. Keep diluted proteins on ice until 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 mTIGIT-biotin + 2 µl distilled water). Add 6 µl of master mixture to every well.

| Component | Blank | Positive Control | Test Inhibitor |
|---|---|---|---|
| 3x Immuno Buffer 1 | 2 µl | 2 µl | 2 µl |
| mCD155-His (32 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 | – | – |
| mTIGIT-biotin (16 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 Immuno Buffer 1 to the well designated “Blank”.
7. Incubate at room temperature for 30 minutes with slow shaking.
8. Thaw mCD155-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: mCD155-His is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.

9. Dilute mCD155-His in 1x Immuno Buffer 1 to 32 ng/µl. Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use.

***Initiating the reaction***

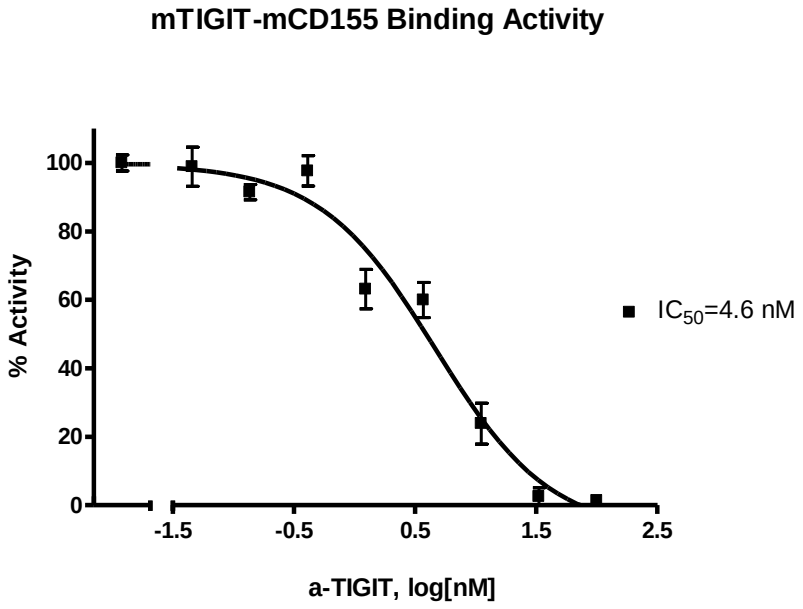
Initiate reaction by adding 2 µl of diluted mCD155-His prepared as described above to each well designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for 60 minutes.

Protect your samples from direct exposure to light. Photobleaching will occur.

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.
2. 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.
3. 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 mTIGIT-biotin or mCD155-His concentrations may improve signal-to-noise ratio.

**Example Results**




*Mouse TIGIT:Mouse CD155 inhibition, measured using the mTIGIT:mCD155 Inhibitor Screening Assay Kit (BPS Bioscience Bioscience #78141) and an anti-TIGIT antibody (BPS Bioscience #71218). Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

**General considerations**

**"Blank" Control:** The "Blank" control is important to determine the background absorbance in the assay.

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

**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.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| Mouse TIGIT, Fc-fusion (IgG1), Avi-tag, Biotin-labeled | #79269 | 25 µg; 50 µg |
| Mouse CD155 (PVR), His-tag | #71167 | 100 µg |
| Mouse CD155 (PVR), His-tag, Biotin-labeled | #71168 | 50 µg |
| 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 |
| Human CD112, His-tag, Biotin-labeled | #71234 | 50 µg |
| Human CD155 (PVR), His-tag | #71181 | 100 µg |
| Human TIGIT:CD155 Homogenous Assay Kit | #72029 | 384 rxns |
| Human TIGIT:CD112 Homogenous Assay Kit | #72030 | 384 rxns |