Description

The CD137:CD137L [Biotinylated] Inhibitor Screening Assay Kit is designed for screening and profiling inhibitors of CD137:CD137L interaction. This kit comes in a convenient 96-well format, with biotin-labeled CD137L, purified CD137, streptavidin-labeled HRP, and assay buffer for 100 reactions. The assay takes advantage of the highly sensitive detection of biotin-labeled CD137L by streptavidin-HRP. First, CD137 is coated on a 96-well plate. Next, CD137L-biotin is incubated with CD137. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can then be measured using a chemiluminescence reader.

Background

CD137, also known as 4-1BB, is a prototype co-stimulatory molecule expressed on T cells. CD137 interaction with its cognate ligand, CD137L, also known as 4-1BBL, results in increased T cell activation and proliferation. Accumulating evidence shows a role for CD137:CD137L signaling in inflammation, suggesting that inhibition of this pathway may provide a therapeutic avenue to treat autoimmune and inflammatory diseases.

Application(s)

- Screen and profile inhibitors of CD137 binding to CD137L.

Supplied Materials

Catalog #	Name	Amount	Storage
71170	CD137 (4-1BB), Fc fusion*	10 µg	-80°C
100239	CD137L (4-1BB ligand), His, Avi, Biotin	2 µg	-80°C
79742	Streptavidin-HRP	10 µl	4°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	4°C
79670	ELISA ECL substrate A (transparent bottle)	6 ml	Room Temperature
	ELISA ECL substrate B (brown bottle)	6 ml	Room Temperature
79699	White 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube*

Materials Required but Not Supplied

Name	Ordering Information
PBS (Phosphate buffered saline) Luminometer or microplate reader capable of reading chemiluminescence Rotating or rocker platform	

Storage Conditions

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

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.

Assay Protocol

All samples and controls should be tested in duplicate.

Day 1 - Coating plate with CD137:

1. Thaw **CD137** on ice. Briefly spin the tube containing **CD137** to recover the full contents of the tube. If the assay plate is going to be used more than once, prepare enough CD137 for this portion of the assay and aliquot the remaining protein into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

   Note: CD137 is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

2. Dilute **CD137** to 2 ng/µl in PBS.
   The concentration of the protein is lot-specific and will be indicated on the tube.

3. Add 50 µl of diluted **CD137** solution to each well and incubate overnight at 4°C. Leave a few wells empty for use as "Uncoated Control".

Day 2 - Blocking and Testing:

1. Dilute **3x Immuno Buffer 1** to 1x Immuno Buffer 1 in distilled water by adding one part stock 3x Immuno Buffer 1 to two parts water.

2. Remove the CD137 solution and wash the plate 3 times with 100 µl of **1x Immuno Buffer 1**. Invert the plate and tap onto clean paper towels to remove the liquid.

3. Block by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature. Invert the plate and tap onto clean paper towels to remove the liquid.

Assay Protocol Part 1:

1. Prepare the Master Mix: N wells × (10 µl of **3x Immuno Buffer 1** + 15 µl of distilled water).

2. Add 25 µl of Master Mix to each well.

3. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   a. If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use distilled water (Diluent Solution).

   OR

   b. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

To prepare serial dilutions of the Test Inhibitor dilute at 10-fold the desired final concentrations using 10% DMSO in distilled water to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in distilled water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: High concentrations of DMSO may interfere with protein binding. If the test inhibitor is dissolved in DMSO, the final DMSO concentration in the assay should be ≤1%.

4. Add 5 µl of diluted inhibitor in the wells designated "Test Inhibitor". For the "Positive Control", "Uncoated Control" and "Blank", add 5 µl of the Diluent Solution (water with or without 10% DMSO).

5. Thaw **CD137L-biotin** on ice. Briefly spin the tube containing the protein to recover the full contents of the tube. If the assay plate is going to be used more than once, prepare enough protein for this portion of the assay and aliquot the remaining **CD137L-biotin** into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

Note: CD137L-biotin is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.

6. Dilute **CD137L-biotin** in **1x Immuno Buffer 1** at 0.5 µg/ml. Keep the diluted protein on ice until use. Discard any unused diluted protein.

7. Add 20 µl of **1x Immuno Buffer 1** to the well designated "Blank".

8. Initiate the reaction by adding 20 µl of diluted **CD137L-biotin** to the wells labeled "Positive Control", "Uncoated Control" and "Test Inhibitor". Incubate at room temperature for two hours.

Component	Blank	Uncoated Control	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl	25 µl
Test inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
CD137L-biotin (5 µg/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

9. Remove the solution. Wash the plate 3 times with 100 µl/well of **1x Immuno Buffer 1**. Invert the plate and tap onto clean paper towels to remove the liquid.

10. Block by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature.

11. Remove the solution. Wash the plate 3 times with 100 µl/well of **1x Immuno Buffer 1**. Invert the plate and tap onto clean paper towels to remove the liquid.

Assay Protocol Part 2:

1. Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer 2**.

2. Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

3. Wash the plate three times with **1x Immuno Buffer 1**. Invert the plate and tap onto clean paper towels to remove the liquid.

4. Block by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Remove the blocking buffer. Tap the plate onto clean paper towels to remove the liquid.

5. Just before use, mix N wells x (50 µl **ELISA ECL Substrate A** and 50 µl **ELISA ECL Substrate B**) and add 100 µl of the mix to each well. Discard any unused chemiluminescent mix.

6. Immediately read in a luminometer or plate reader capable of reading chemiluminescence. The "Blank" value is subtracted from all readings.

Reading Chemiluminescence:
Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and not spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without binding partner (typically we set this value as 100).

Example Results

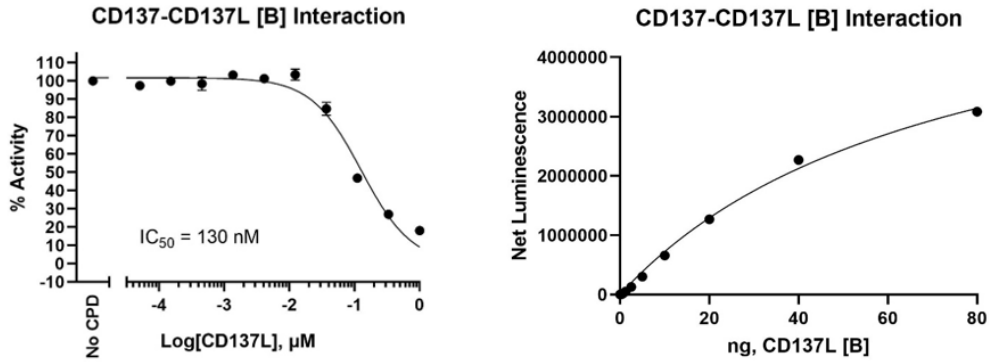

Figure 1: CD137:CD137L[Biotinylated] Interaction Binding and Inhibition.
CD137:CD137L[Biotinylated] binding (right) and inhibition of binding (left), measured using the CD137:CD137L[Biotinylated] Inhibitor Screening Assay Kit (BPS Bioscience #78590). Luminescence was measured using a Bio-Tek fluorescent microplate reader.

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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

References

1. Drenkard D. *et al., FASEB J.* 2007; **21**: 456-463
2. Kwon B. *et al., Immune Netw.* 2009; **9(3)**: 84-89.

Related Products

Products	*Catalog #*	*Size*
CTLA4:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72209	96 reactions
CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72207	96 reactions
PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit	72003	96 reactions
PD-1:PD-L2[Biotinylated] Inhibitor Screening Assay Kit	72004	96 reactions