



6042 Cornerstone Court W, Ste B  
San Diego, CA 92121  
**Tel:** 1.858.202.1401  
**Fax:** 1.858.481.8694  
**Email:** info@bpsbioscience.com

## Data Sheet

### ***CD137[Biotinylated]:CD137L Inhibitor Screening Assay Kit***

**Catalog # 72025**

**Size: 96 reactions**

**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 s 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.

**DESCRIPTION:** The *CD137:CD137L 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 CD137, purified CD137L, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled CD137 by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, CD137L is coated on a 96-well plate. Next, CD137-biotin is incubated with CD137L on the plate. 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.

#### **COMPONENTS:**

<b>Catalog #</b>	<b>Component</b>	<b>Amount</b>	<b>Storage</b>	
71171	CD137 (4-1BB), His-tagged, Biotin-labeled	2 µg	-80°C	<b>(Avoid freeze/thaw cycles!)</b>
71189	CD137L (4-1BB ligand), His tagged	10 µg	-80°C	
79742	Streptavidin-HRP	15 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 Temp.	
	ELISA ECL substrate B (brown bottle)	6 ml	Room Temp.	
79699	White 96-well microplate	1	+4°C	

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**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

PBS (Phosphate buffered saline)

Luminometer or fluorescent microplate reader capable of reading chemiluminescence

Rotating or rocker platform

**APPLICATIONS:** This kit is useful for screening for inhibitors of CD137 binding to CD137L.

**STABILITY:** One year from date of receipt when stored as directed.

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

**ASSAY PROTOCOL:**

All samples and controls should be tested in duplicate.

**Coating the plate with CD137L:**

- 1) Thaw **CD137L** on ice. Upon first thaw, briefly spin tube containing **CD137L** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining CD137L in aliquots at -80°C. *Note: CD137L is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*
- 2) Dilute **CD137L** to 2 ng/μl in PBS.
- 3) Add 50 μl of diluted **CD137L** solution to each well and incubate overnight at 4°C. Leave a couple of wells empty (uncoated), for use with the "Ligand Control" (see below).
- 4) Dilute **3x Immuno Buffer 1** to **1x Immuno Buffer 1** in water.
- 5) Decant to remove supernatant. Wash the plate 3 times with 100 μl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
- 6) Block wells by adding 100 μl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature. Remove supernatant as described in step 4.

**Step 1:**

- 1) Prepare the master mixture: N wells × (10 μl **3x Immuno Buffer 1** + 15 μl H<sub>2</sub>O).
- 2) Add 25 μl of master mixture to each well. Use uncoated wells for the "Ligand Control."

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- 3) Add 5  $\mu$ l of inhibitor solution to each well designated “Test Inhibitor.” For the “Positive Control,” “Ligand Control” and “Blank,” add 5  $\mu$ l of 10% DMSO in water (inhibitor buffer).

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer 1	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
H <sub>2</sub> O	15 $\mu$ l	15 $\mu$ l	15 $\mu$ l	15 $\mu$ l
Test Inhibitor/Activator	–	–	–	5 $\mu$ l
10% DMSO in water (Inhibitor buffer)	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l	–
1x Immuno Buffer 1	20 $\mu$ l	–	–	–
CD137-biotin (0.5 ng/ $\mu$ l)	–	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l
<b>Total</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>

- 4) Thaw **CD137-biotin** on ice. Upon first thaw, briefly spin tube containing protein to recover full contents of the tube. Aliquot **CD137-biotin** into single use aliquots. Immediately store remaining undiluted protein in aliquots at -80°C. *Note: CD137-biotin is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
- 5) Dilute **CD137-biotin** in **1x Immuno Buffer 1** at 0.5 ng/ $\mu$ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.
- 6) Add 20  $\mu$ l of **1x Immuno Buffer 1** to the well designated “Blank.”
- 7) Initiate reaction by adding 20  $\mu$ l of diluted **CD137-biotin** (see Step 1-5) to wells labeled “Positive Control,” “Ligand Control” and “Test Inhibitor.” Incubate at room temperature for two hours.
- 8) Decant to remove supernatant. Wash the plate 3 times with 100  $\mu$ l/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
- 9) Block wells by adding 100  $\mu$ l of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-8.

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### Step 2:

- 1) Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer 2**.
- 2) Add 100  $\mu$ l to each well. Incubate for 1 hour at room temperature with slow shaking.
- 3) Wash plate three times with **1x Immuno Buffer 1**. Tap onto clean paper towels to remove liquid.
- 4) Block wells by adding 100  $\mu$ l of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.
- 5) Just before use, mix on ice 50  $\mu$ l **ELISA ECL Substrate A** and 50  $\mu$ l **ELISA ECL Substrate B** per well of the reaction, then add 100  $\mu$ l to each well. Discard any unused chemiluminescent reagent after use.
- 6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "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 is not emission 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).

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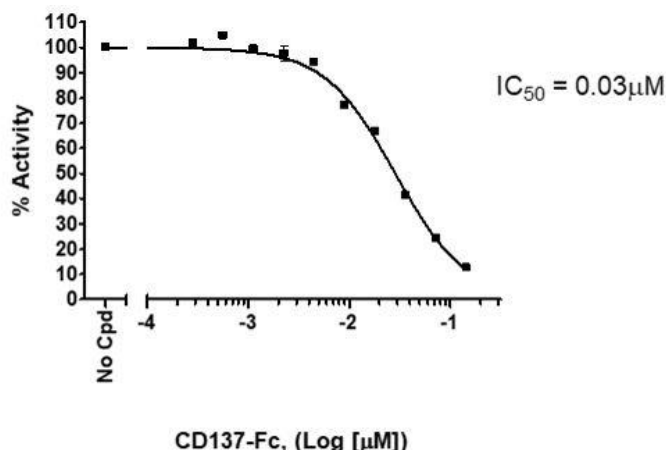
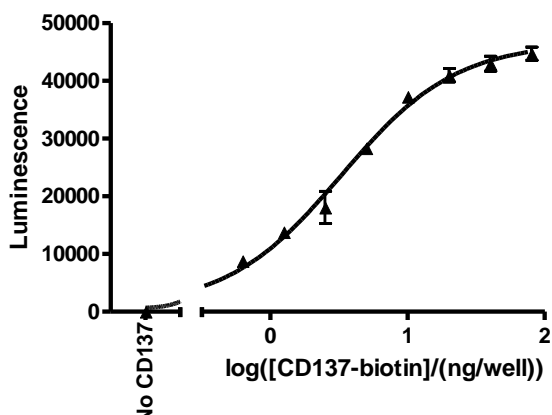
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**Example of Assay Results:**



CD137[Biotinylated]:CD137L binding (left) and inhibition (right), measured using the using the *CD137[Biotinylated]:CD137L Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog # 72025. Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com).*

**RELATED PRODUCTS:**

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
CD137 (4-1BB), Fc fusion	71170	100 µg
CD137 (4-1BB), His-tagged, Biotin-labeled	71171	50 µg
CD137L (4-1BB ligand), His-tagged	71189	100 µg
OX40, Fc fusion	71175	100 µg
OX40L, Fc fusion	71185	100 µg
CTLA4:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72009	96 rxns
CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72007	96 rxns
PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit	72003	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Assay Kit	72004	96 rxns
PD-1	71106	100 µg
PD-1, Biotin labeled	71109	50 µg
PD-L1	71104	100 µg
PD-L1, Biotin-labeled	71105	50 µg
PD-L2	71107	100 µg
PD-L2, Biotin-labeled	71108	50 µg

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## TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	CD137 or CD137L has lost binding capacity.	Protein loses activity upon repeated freeze/thaw cycles. Use fresh CD137-biotin, (BPS Bioscience #71171) and fresh CD137L (BPS Bioscience #71189). Store proteins in single-use aliquots. Increase time of protein incubation. Increase protein concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
Luminescent signal is erratic or varies widely among wells	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Insufficient washes	Increase number of washes. Increase wash volume. Add Tween-20 to 0.1% in washing buffer.
Background (signal to noise ratio) is high	Sample solvent is inhibiting the protein	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of protein incubation.
	Results are outside the linear range of the assay	Use different concentrations of CD137-biotin (BPS Bioscience #71171) to create a standard curve.

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