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Notch1:DLL4 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit

#82284
96 reactions

Description

The Notch1:DLL4 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is designed for screening and profiling molecules that block the binding of Notch1 (neurogenic locus notch homolog protein 1) and DLL4 (delta like canonical notch ligand 4). This kit comes in a convenient 96-well format, with enough recombinant purified Notch1, biotin-labeled DLL4, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled DLL4 by streptavidin-HRP.

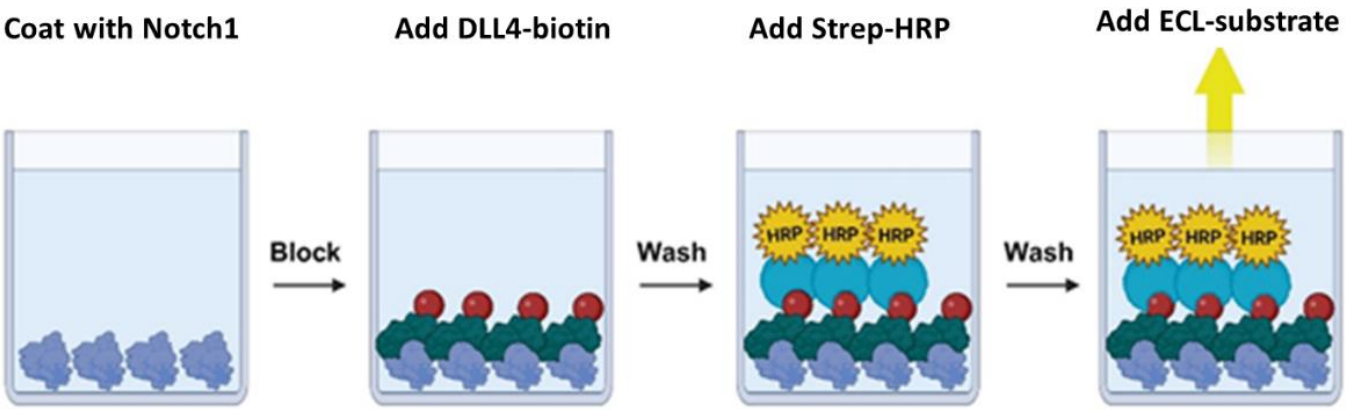

Figure 1: Illustration of the mechanism of Notch1:DLL4 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.

Notch1 is coated onto a 96-well plate. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. After incubation with Biotin-DLL4, the plate is washed and Streptavidin-HRP is added. After the ELISA ECL substrate is added the resulting signal can be measured using a chemiluminescence microplate reader. The chemiluminescence signal is proportional to the binding of Notch1 to DLL4.

Background

The Notch (neurogenic locus notch homolog protein) signaling is evolutionally highly conserved and implicated in various aspects of cancer development and progression. Aberrant activation of Notch1 signaling, often through mutations or overexpression, is associated with several types of cancer, including leukemia, breast cancer, lung cancer, and others. DLL4 (delta like canonical notch ligand 4), as a ligand of Notch receptors, can also influence tumor angiogenesis, promoting the growth of blood vessels within tumors and facilitating tumor progression. As Notch1 and DLL4 signaling pathway plays significant roles in cancer development, several Notch1 and DLL4 neutralizing antibodies have been evaluated in preclinical studies and clinical trials across various cancer types.

Application(s)

Screening small molecule inhibitors or antibodies that block Notch1 binding to DLL4.

Supplied Materials

Catalog #	Name	Amount	Storage
101904	Human DLL4, Fc-Avi-Tag, Biotin-Labeled *	10 µg	-80°C
101897	Human Notch1, Avi-His-Tag*	10 µg	-80°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79742	Streptavidin-HRP	10 µl	+4°C
79670	ELISA ECL Substrates A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrates B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

**The initial concentration of both DLL4 and Notch1 is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- 1x PBS buffer (Phosphate Buffer Saline)
- Microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** 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

The DMSO concentration in the final reaction should be ≤1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Non-Coated Condition”, “Blank”, “Positive Control” and “Test Compound” wells.
- We recommend preincubating antibodies or protein inhibitors with the target protein prior to the addition of the binding partner.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

- Anti-Notch1 Neutralizing Antibody (#102065) may be used as internal control. If not running a dose response curve, we recommend running the antibody at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

Step 1 - Plate coating with Notch1 protein

Coat the plate one day prior to running your samples.

1. Thaw **Notch1** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **Notch1** protein to 2 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **Notch1** protein solution to each well.
4. Add 50 µl of PBS to the "Non-Coated Condition" wells.
5. Incubate at 4°C overnight.
6. Prepare **1x Immuno Buffer** by diluting 3-fold **3x Immuno Buffer 1** with distilled water.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.
9. Tap the plate onto clean paper towel to remove the liquid.
10. Add 100 µl of Blocking Buffer 2 to every well.
11. Incubate for 1 hour at Room Temperature (RT) with gentle agitation.
12. Tap the plate onto clean paper towel to remove the liquid.
13. Start your testing immediately.

Step 2.1: Assessment of the inhibition/blocking of Notch1 binding to DLL4 by anti-Notch1 antibodies.

1. Prepare a serial dilution of **anti-Notch** antibody or Notch1-directed blocker of interest in Blocking Buffer 2 at the desired concentrations (50 µl/well).
2. Add 50 µl of the diluted antibody/blocker to the "Test Compound" wells.
3. Add 100 µl of Blocking Buffer 2 to the "Blank" wells.
4. Add 50 µl of Blocking Buffer 2 to the "Positive Control" wells.
5. Incubate the plate for 30 minutes (up to 1 hour) at RT with gentle agitation.

6. Thaw the **DLL4-Biotin** on ice. Briefly spin the tube to recover the full content.
7. Dilute **DLL4-Biotin** to 1.5 µg/ml with Blocking Buffer 2 (50 µl/well).
8. Add 50 µl of diluted **DLL4-Biotin** to the "Test Compound" and "Positive Control" wells.
9. Incubate the plate at RT for 1 hour with gentle agitation.

	Blank	**Positive Control**	**Test Compound**
Blocking Buffer 2	100 µl	50 µl	-
Test Compound	-	-	50 µl
Diluted DLL4-Biotin (1.5 µg/ml)	-	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

10. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.
11. Tap the plate onto clean paper towel to remove the liquid.
12. Block the wells by adding 100 µl of Blocking Buffer 2 to every well and incubate for 10 minutes.
13. Tap the plate onto clean paper towel to remove the liquid.

Step 3.1: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 2 (100 µl/well).
2. Add 100 µl of the diluted Streptavidin-HRP to each well.
3. Incubate the plate for 1 hour at RT with gentle agitation.
4. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
7. Add 100 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.
8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.
9. The "Blank" value should be subtracted from all readings.

Step 2.2: Assessment of the inhibition/blocking of Notch1 binding to DLL4 by small molecules.

1. 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.

   1.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water at concentrations 10-fold higher than the desired final concentrations.

   For the positive and negative controls use distilled water (Diluent Solution).

   OR

   1.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using distilled water containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   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: The final concentration of DMSO in the assay should not exceed 1%.

2. Add 5 µl of diluted Test Inhibitor to each well labeled "Test Compound".

3. Add 5 µl of the Diluent Solution to the "Positive Control" and "Blank" wells.

4. Thaw **DLL4-Biotin** on ice. Briefly spin the tube to recover the full content.

5. Dilute DLL4-Biotin to 1.5 µg/ml with Blocking Buffer 2 (20 µl/well).

6. Add 20 µl of diluted DLL4-Biotin to the wells labeled "Test Compound" and "Positive Control".

7. Add 25 µl of Blocking Buffer 2 to the "Test Inhibitor" and "Positive Control" wells.

8. Add 45 µl of Blocking Buffer 2 to the "Blank" wells.

9. Incubate the plate at RT for 1 hour with gentle agitation.

	Blank	**Positive Control**	**Test Compound**
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted DLL4-Biotin (1.5 µg/ml)	-	20 µl	20 µl
Blocking Buffer 2	45 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

10. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.

11. Block the wells by adding 100 µl of Blocking Buffer 2 to every well and incubate for 10 minutes at RT.

12. Tap the plate onto clean paper towel to remove the liquid.

Step 3.2: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 2 (100 µl/well).

2. Add 100 µl of the diluted Streptavidin-HRP to each well.

3. Incubate the plate for 1 hour at RT with gentle agitation.

4. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.

5. Tap the plate onto clean paper towel to remove the liquid.

6. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

7. Add 100 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.

8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.

9. The “Blank” value should be 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 enzyme (typically we set this value as 100).

Example Results

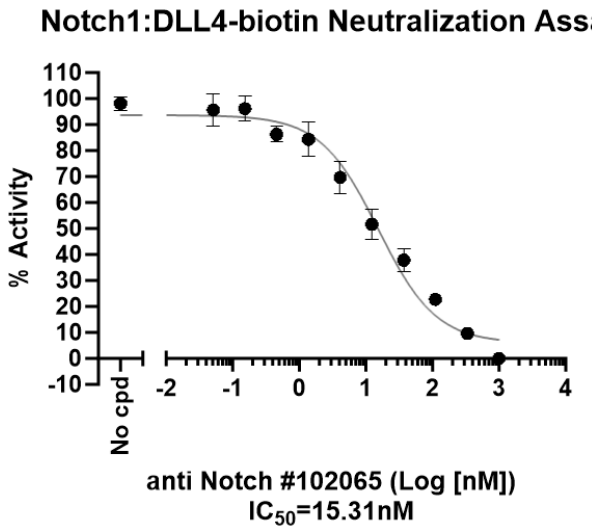

Figure 1. Inhibition of Notch1:DLL4 binding by Anti-Notch Neutralizing Antibody.
Notch1:DLL4 binding was evaluated in the presence of increasing concentrations of Anti-Notch Neutralizing Antibody (#102065). Results are expressed as percent activity, in which the binding activity in the absence of inhibitor is set to 100%.

Data shown is representative. 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.

Related Products

Products	*Catalog #*	*Size*
Anti-Notch Neutralizing Antibody	102065	50 µg/100 µg
Notch1, Avi-His-Tag (Human)	101897	20 µg/100 µg
DLL4, Fc-Avi-Tag, Biotin-Labeled (Human)	101904	10 µg/50 µg
DLL4, Fc-Avi-Tag (Human)	101903	10 µg/50 µg

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