Description

The NSD3 Chemiluminescent Assay Kit is designed to measure the activity of NSD3 (Nuclear receptor-binding SET domain 3) complex activity for screening and profiling applications. The NSD3 assay kit comes in a convenient 96-well format, with enough recombinant purified NSD3 (amino acids 1021-1322), primary and secondary antibodies, S-adenosylmethionine (SAM), pre-coated plate with RNA substrate, blocking buffer and detection reagents for 100 enzyme reactions.

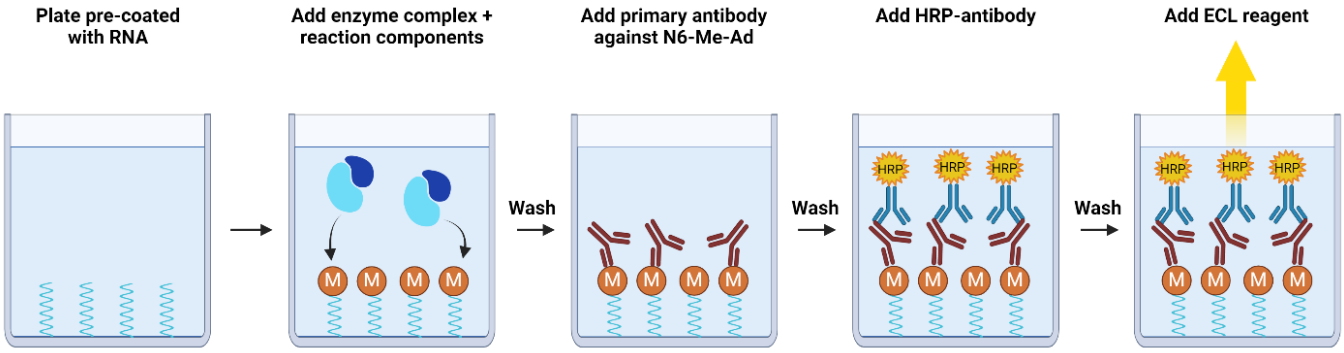

Figure 1: Illustration of the NSD3 Chemiluminescent Assay Kit assay principle.

Background

NSD3 (Nuclear receptor-binding SET domain 3), also known as WHSC1L1 (Wolf-Hirschhorn syndrome candidate 1-like 1), is a member of the NSD histone methyltransferase family of proteins, and it has 3 isoforms: NSD3L (long version), NSD3S (short version) and WHISTLE (WHSC1-like 1 isoform 9 with methyltransferase activity to lysine). It methylates histone H3 at lysine 36, via the formation of a complex with LSD2 (lysine-specific demethylase 2) and G9a/EHMT2 (euchromatic histone lysine methyltransferase 2). In addition to methylating histones, it can also methylate NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). NSD3 is ubiquitously expressed. The gene is often amplified in cancer, such as in breast, lung, colon and lung cancer. Mutations and fusion proteins are also frequent in cancer. Its role in cancer have made it an attractive target in cancer therapy. Inhibitors have been developed targeting the PWWP1 (proline-tryptophan-tryptophan-proline) domain of the protein, such as BI-9321, which has been shown to decrease cancer cell proliferation. More recently, NSD3 PROTACs were able to degrade NSD3 in cancer cell lines of MM (multiple myeloma) and lung cancer. A better understanding of the roles of this protein and the development of isoform specific inhibitors may open new therapeutic avenues in cancer treatment.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
51036	NSD3 (1021-1322) (WHSC1L1), GST-Tag*	25 µg	-80°C
	100 µM S-Adenosylmethionine	250 µl	-80°C
52140P2	Primary Antibody 16-2	12.5 µl	-80°C
52193Z	4x HMT Assay Buffer 7	3 ml	-20°C
52100	Blocking Buffer 4	50 ml	+4°C
52131H	Secondary HRP-Labeled Antibody 2	10 µl	-80°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp.
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp.
	96-well plate precoated with the substrate	1	+4°C

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

Materials Required but Not Supplied

- TBST Buffer (1x Tris-buffered saline (TBS), pH 8 containing 0.05% Tween-20)
- Luminometer or 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 is compatible with up to 1% final DMSO concentration.
- Use RNAse-free conditions.
- Avoid strong acids or bases, ionic detergents or high salt conditions.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

- We recommend using Chaetocin (#27221) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1:

1. Rehydrate the microwells by adding 150 µl of TBST Buffer to every well.

2. Incubate 15 minutes at Room Temperature (RT).

3. Tap the strip onto clean paper towels to remove liquid.

4. Thaw **S-adenosylmethionine** on ice. Upon first thaw, briefly spin tube containing S-adenosylmethionine to recover full content of the tube.

   Note: Store remaining S-adenosylmethionine in single use aliquots (5 µl minimum volume per aliquot) at -80°C immediately.

5. Prepare a Master Mixture (25 µl/ well): N wells × (7.5 µl of **4x HMT Assay Buffer 7** + 2.5 µl of 100 µM **S-adenosylmethionine** + 15 µl of distilled water).

6. Add 25 µl of Master Mixture to each well.

7. Thaw **NSD3 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube.

8. Dilute 4-fold the 4x HMT Assay Buffer 7 with distilled water. This makes 1x HMT Assay Buffer 7.

9. 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.

   9.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration in 1x HMT Assay Buffer 7.

   For the positive and negative controls, use 1x HMT Assay Buffer 7 (Diluent Solution).

   OR

   9.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x HMT Assay Buffer 7 (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x HMT Assay Buffer 7 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x HMT Assay Buffer 7 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

10. Dilute **NSD3 enzyme** in **1x HMT Assay Buffer 7** to 12.5 ng/µl (20 µl/ well).

11. Add 5 µl of inhibitor solution of each well designated "Test Inhibitor".

12. For the "Positive Control," and "Blank," add 5 µl of **1x HMT Assay Buffer 7** in Diluent Solution.

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

14. Initiate reaction by adding 20 µl of diluted **NSD3** (prepared as described above) to the wells labeled "Test Inhibitor" and "Positive Control".

15. Incubate 2 hours at RT on a rotating platform.

	Blank	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x HMT Assay Buffer 7	20 µl	-	-
Diluted NSD3 (12.5 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

16. Wash the wells three times with 200 µl of TBST Buffer.

17. Blot dry onto clean paper towels.

18. Add 100 µl of **Blocking Buffer 4** to every well.

19. Shake on a rotating platform for 10 minutes. Remove supernatant as above.

Step 2:

1. Dilute 800-fold the **Primary Antibody 16-2** with **Blocking Buffer 4** (100 µl/ well).

2. Add 100 µl of diluted Primary Antibody 16-2 per well.

3. Incubate 1 hour at RT with slow shaking.

4. Wash the plate three times with 200 µl of TBST Buffer.

5. Blot dry onto clean paper towels.

6. Add 100 µl of **Blocking Buffer 4** to every well.

7. Shake on a rotating platform for 10 minutes. Remove supernatant as above.

Step 3:

1. Dilute 1,000-fold the **Secondary HRP-Labeled Antibody 2** with **Blocking Buffer 4** (100 µl/ well).

2. Add 100 µl per well.

3. Incubate for 30 minutes at RT with slow shaking.

4. Wash the plate three times with 200 µl of TBST Buffer.

5. Blot dry onto clean paper towels.

6. Add 100 µl of **Blocking Buffer 4** to every well.

7. Shake on a rotating platform for 10 minutes. Remove supernatant as above.

8. Just before use, mix on ice 50 µl of **ELISA ECL substrate A** and 50 µl of **ELISA ECL substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

9. Immediately read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

10. 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

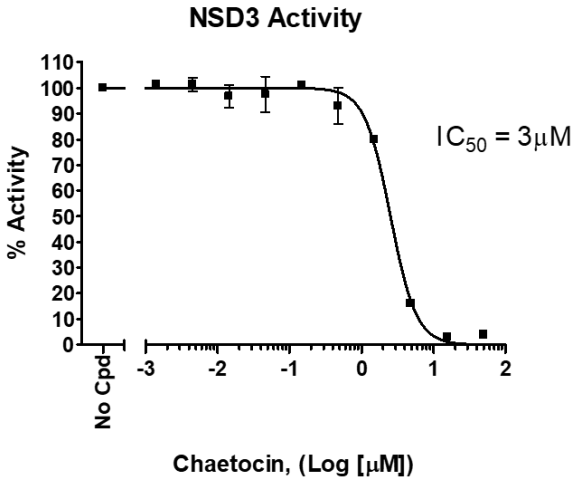

Figure 1: Inhibition of NSD3 activity by Chaetocin.
NSD3 was incubated with increasing concentrations of chaetocin (#27221). Chemiluminescence was measured using a Bio-Tek microplate reader.

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

References

Dillon S.C., *et al*., 2005 *Genome Biology* 6:227.
Bennet R.L., *et al.,* 2017 *Cold Spring Harb Perspect Med.* 7(6): a026708.
Nunez Y., *et al.*, 2024 *Int J Mol Sci* 25(2): 944.

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*
NSD3 Chemiluminescence Assay Kit	79358	384 reactions
NSD2 Chemiluminescence Assay Kit	79359	384 reactions
NSD1 Chemiluminescence Assay Kit	53010	96 reactions
NSD2 (catalytic domain), GST-tag Recombinant	51026	50 µg
NSD1, GTS-tag Recombinant	51024	50 µg

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