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Data Sheet

TNKS1 Histone Ribosylation Assay Kit (Biotin-labeled NAD+)
Catalog # 80579

DESCRIPTION: The TNKS1 Histone Ribosylation Assay Kit (Biotin-labeled NAD+) is designed to measure Tankyrase 1 (TNKS1) activity for screening and profiling applications. TNKS1 catalyzes the NAD-dependent addition of poly(ADP-ribose) to the substrate proteins. The TNKS1 assay kit comes in a convenient 384-well format, with purified TNKS1 enzyme, histone mixture, and PARP assay buffer for 384 enzyme reactions. The key to the TNKS1 Histone Ribosylation Assay (Biotin-labeled NAD+) is the biotinylated substrate. With this kit, only three simple steps are required for TNKS1 reactions. First, histone proteins are coated on a 384-well plate. Next, the biotinylated TNKS1 substrate is incubated with an assay buffer that contains the TNKS1 enzyme. Finally, the plate is treated with streptavidin-HRP followed by addition of the HRP substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage
80504	TNKS1	2 x 5 µg	-80°C
52029	5x histone mixture	2 x 1 ml	-80°C
80601	10x Assay Mixture Containing Biotinylated Substrate	2 x 300 µl	-80°C
80602	10x PARP assay buffer	2 x 1 ml	-20°C
79743	Blocking buffer 3	2 x 25 ml	+4°C
80611	Streptavidin-HRP	2 x 100 µl	+4°C
79670	ELISA ECL substrate A (translucent bottle)	2 x 6 ml	Room Temperature
	ELISA ECL substrate B (brown bottle)	2 x 6 ml	Room Temperature
78188	384-well plate	1	Room Temperature

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
1x PBS buffer
PBST buffer (1x PBS, containing 0.05% Tween-20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

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APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.
STABILITY: Up to 1 year when stored as recommended.

REFERENCE:
Brown, J.A., Marala, R.B. *J. Pharmacol. Toxicol. Methods* 2002 **47:**137-41.

Assay Protocol:
All samples and controls should be tested in duplicate.

Coating the plate with the histone mixture:

1) Dilute 5x histone mixture 1:5 in PBS.

2) Add 25 µl diluted histone mixture to each well and incubate overnight at 4°C.

3) Wash the plate 3 times with 100 µl PBST buffer.

4) Block the wells by adding 100 µl of Blocking buffer 3 to every well. Incubate for 60 minutes at room temperature.

5) Wash the plate 3 times with 100 µl PBST buffer

(Alternatively, the plate can be coated for 90 minutes at 37°C followed by 60 minutes blocking at room temperature. All washing steps should be the same.)

Step 1: Ribosylation reaction

1) Prepare the master mixture: N wells x (1.25 µl **10x PARP assay buffer** + 1.25 µl **10x PARP assay mixture** + 10 µl H_2O)

2) Thaw TNKS1 **enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **TNKS1 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note:* **TNKS1 *enzyme*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute **TNKS1 enzyme** in **1X PARP assay buffer** at 1.25 – 2.5 ng/µl (12.5 - 25 ng/10 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

   Add 12.5 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the "Substrate Control", add 1.25 µl **10x PARP assay buffer** + 11.25 µl H_2O.

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	Blank	Positive Control	Substrate Control	Test Inhibitor
10X PARP Assay Buffer	1.25 µl	1.25 µl	1.25 µl	1.25 µl
10X assay mixture	1.25 µl	1.25 µl	–	1.25 µl
H_2O	10 µl	10 µl	11.25 µl	10 µl
Test Inhibitor	–	–	–	2.5 µl
Inhibitor buffer (no inhibitor)	2.5 µl	2.5 µl	2.5 µl	
1x PARP buffer	10 µl	-	-	-
TNKS1 (~ 2 ng/µl)	–	10 µl	10 µl	10 µl
Total	25 µl	25 µl	25 µl	25 µl

4) Add 2.5 µl of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control”, “Substrate Control”, and ”Blank”, add 2.5 µl of the same solution without inhibitor (inhibitor buffer).

5) Add 10 µl of **1x PARP buffer** to the well designated “Blank”.

6) Initiate the reactions by adding 10 µl of diluted **TNKS1** prepared as described above. Incubate the reactions for 1 hour at room temperature.

7) Wash the plate 3 times with 100 µl PBST per well.

Step 3: Detection

1) Dilute **Streptavidin-HRP** 1:50 in Blocking buffer 3.

2) Add 25 µl of diluted **Streptavidin-HRP** to each well. Incubate for 30 minutes at room temperature.

3) Wash three times with 100 µl PBST buffer as above.

4) Just before use, mix on ice 25 µl **ELISA ECL substrate A** and 25 µl **ELISA ECL substrate B** and add 50 µl per well. Discard any unused chemiluminescent reagent after use.

5) Incubate for 5 minutes at room temperature then read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

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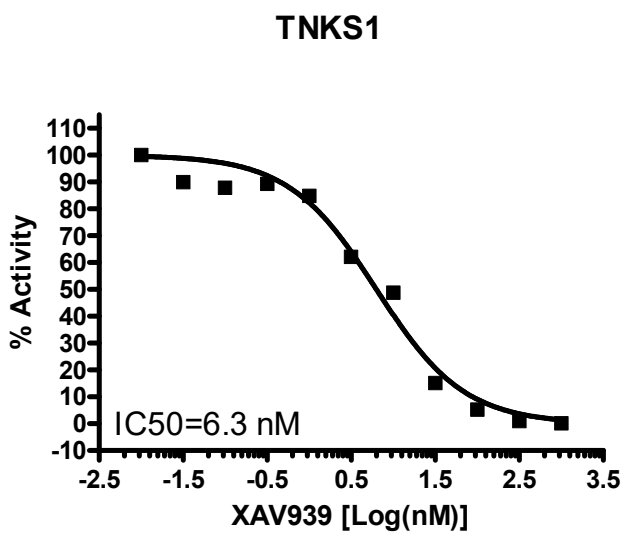
Example of Assay Results:



Inhibition of TNKS1 enzyme (Cat. # 80504) with XAV939 (Cat. # 27100), measured using the TNKS1 Histone Ribosylation Assay Kit (Biotin-labeled NAD+), BPS Bioscience (Cat. # 80579). 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*

RELATED PRODUCTS:

Product	Cat. #	Size
TNKS1 Histone Ribosylation Assay Kit (Antibody Detection)	80574	96 rxns.
TNKS2 Histone Ribosylation Assay Kit (Antibody Detection)	80576	96 rxns.
TNKS2 Histone Ribosylation Assay Kit (Biotin-labeled NAD+)	80578	96 rxns.
TNKS1 (PARP5A) Enzyme	80504	10 µg
TNKS2 (PARP5B), (667-end) Enzyme	80505	10 µg
TNKS2 (PARP5B), (849-end) Enzyme	80515	10 µg
PARP5b (TNKS2) Assay Kit	80576	96 rxns.
PARP6 Assay Kit	80556	32 rxns.
PARP1 Enzyme	80501	10 µg
PARP2 Enzyme	80502	10 µg
PARP3 Enzyme	80503	10 µg
PARP6 Enzyme	80506	10 µg