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Data Sheet

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

Catalog # 80573

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 96-well format, with purified TNKS1 enzyme, histone mixture, and PARP assay buffer for 100 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 96-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:

Reagent	Amount	Storage
TNKS1 (BPS # 80504)	5 µg	-80°C
5x histone mixture	1 ml	-80°C
10x Assay Mixture containing biotinylated substrate	300 µl	-80°C
10x PARP assay buffer	1 ml	-20°C
Blocking buffer	25 ml	+4°C
Streptavidin-HRP	100 µl	+4°C
HRP chemiluminescent substrate (2 components)	6 ml each	+4°C
96-well module	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

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

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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 50 µl diluted histone mixture to each well and incubate overnight at 4°C.

3) Wash the plate 3 times with 200 µl PBST buffer.

4) Block the wells by adding 150 µl of Blocking buffer to every well. Incubate for 60 minutes at room temperature.

5) Wash the plate 3 times with 200 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 (2.5 µl **10x PARP assay buffer** + 2.5 µl **10x PARP assay mixture** + 20 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 1.5 – 2.5 ng/µl (30 - 50 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

Add 25 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the "Substrate Control", add 2.5 µl **10x PARP assay buffer** + 22.5 µl **H_2O.**

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	Blank	Positive Control	Substrate Control	Test Inhibitor
10X PARP Assay Buffer	2.5 µl	2.5 µl	2.5 µl	2.5 µl
10X assay mixture	2.5 µl	2.5 µl	–	2.5 µl
H_2O	20 µl	20 µl	22.5 µl	20 µl
Test Inhibitor	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	
1x PARP buffer	20 µl	-	-	-
TNKS1 (~ 2 ng/µl)	–	20 µl	20 µl	20 µl
Total	50 µl	50 µl	50 µl	50 µl

4) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control", and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer).

5) Add 20 µl of 1x PARP buffer to the well designated "Blank".

6) Initiate the reactions by adding 20 µl of diluted TNKS1 prepared as described above. Incubate the reactions for 1 hour at room temperature.

7) Wash the plate 3 times with 200 µl PBST per well.

Step 3: Detection

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

2) Add 50 µl of diluted **Streptavidin-HRP** to each well. Incubate for 30 minutes at room temperature.

3) Wash three times with 200 µl PBST buffer as above.

4) Just before use, mix on ice 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

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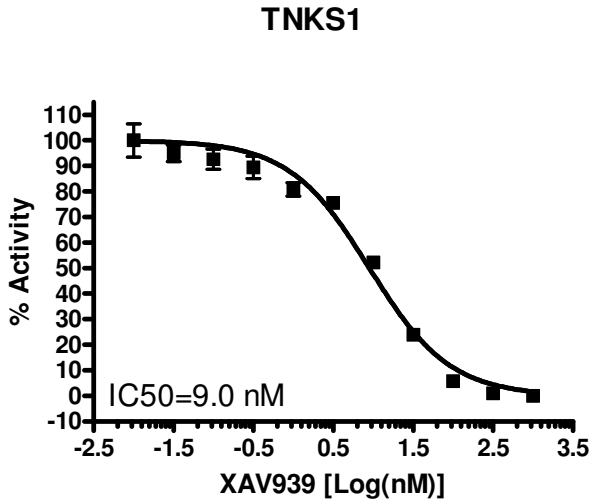
5) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavenlength 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 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. # 80573). 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

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RELATED PRODUCTS:

Product	Cat. #	Size
PARP2 Assay Kit	#80552	96 rxns.
PARP3 Assay Kit	#80553	96 rxns.
TNKS1 Histone Ribosylation Assay Kit (Antibody Detection)	#80574	96 rxns.
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
TNKS1 (PARP5A) Enzyme	#80504	10 µg
TNKS2 (PARP5B), (667-end) Enzyme	#80505	10 µg
TNKS2 (PARP5B), (849-end) Enzyme	#80515	10 µg
PARP7 Enzyme	#80507	10 µg
PARP9 Enzyme	#80509	10 µg
PARP11 Enzyme	#80511	10 µg
PARP12 Enzyme	#80512	10 µg