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Data Sheet

TNKS1 Histone Ribosylation Colorimetric Assay Kit

Catalog: #80582

DESCRIPTION: The *TNKS1 Histone Ribosylation Colorimetric Assay Kit* 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 is the biotinylated NAD+ 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 NAD+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 colorimetric HRP substrate to produce color that can then be measured using a UV/Vis spectrophotometer microplate reader.

COMPONENTS:

Catalog #	Reagent	Amount	Storage
80504	TNKS1	5 µg	-80°C
52029	5x histone mixture	1 ml	-80°C
80601	10x Assay Mixture containing biotinylated substrate	300 µl	-80°C
80602	10x PARP assay buffer	1 ml	-20°C
79743	Blocking buffer 3	25 ml	+4°C
80611	Streptavidin-HRP	100 µl	+4°C
	Colorimetric HRP substrate	10 ml	+4°C
79964	Transparent 96-well plate	1	Room Temp.

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

1x PBS buffer
PBST buffer (1x PBS, containing 0.05% Tween-20)
2 M sulfuric acid (aqueous)
Rotating or rocker platform
Adjustable micropipettor and sterile tips
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*

*Alternately, a spectrophotometer reading at 650 nm may be used, but sensitivity of the assay will be greatly reduced.

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.

Step 1: 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 three times with 200 µl PBST buffer.

4) Block the wells by adding 150 µl of **Blocking buffer 3** to every well. Incubate for 60 minutes at room temperature.

5) Wash the plate three 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 2: 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 distilled water)

2) 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 distilled water.

3) 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).

4) Prepare 1x PARP buffer by adding 1 part of **10x PARP Assay buffer** to 9 parts distilled water (v/v). Prepare only enough **1x PARP buffer** for the assay. Add 20 µl of **1x PARP buffer** to the wells designated "Blank".

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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
Distilled water	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

5) 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.*

6) 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.

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

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

Step 3: Detection

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

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) Add 100 µl of the **Colorimetric HRP Substrate** to each well and incubate the plate at the room temperature until blue color is developed in the positive control well. For TNKS1, it normally takes 15~20 min to fully develop the color. However, the optimal incubation time may vary, and should be determined empirically by the user.

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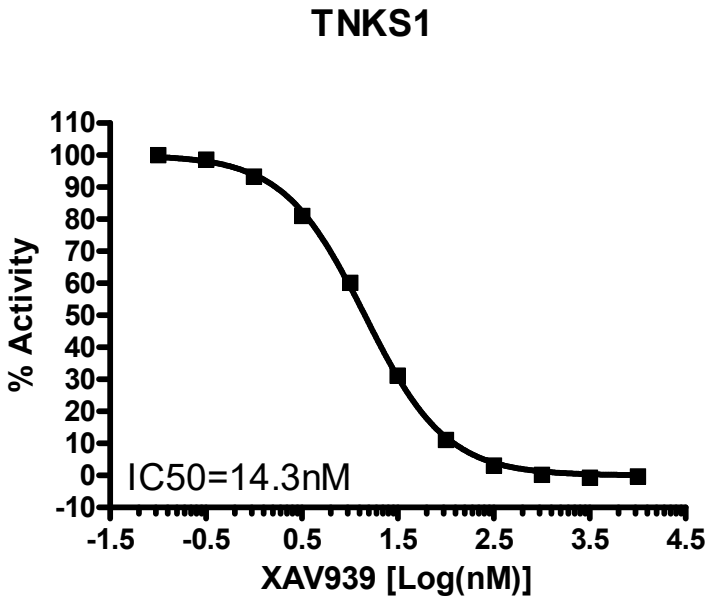
5) After the blue color is developed, add 100 µl of 2 M sulfuric acid to each well. Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader. The blank wells should exhibit an absorbance of ~ 0.05 at 450 nm. *Alternatively, the plate may be read at 650 nm without adding 2 M sulfuric acid, but the Signal-to-Background ratio will be decreased.*

Example of Assay Results:



Inhibition of TNKS1 enzyme (Cat. #80504) with XAV939 (Cat. #27100), measured using the TNKS1 Histone Ribosylation Colorimetric Assay Kit, BPS Bioscience (Cat. #80582). Absorbance at 450 nm was measured using a Tecan 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 Name	Catalog #	Size
PARP1 Colorimetric Assay Kit	80580	96 rxns
PARP2 Colorimetric Assay Kit	80581	96 rxns
TNKS2 Histone Ribosylation Colorimetric Assay Kit	80583	96 rxns
TNKS1 Histone Ribosylation Assay Kit	80573	96 rxns
TNKS1 Histone Ribosylation Kit (Antibody Detection)	80575	96 rxns
TNKS2 Histone Ribosylation Assay Kit	80578	96 rxns
TNKS2 Histone Ribosylation Kit (Antibody Detection)	80576	96 rxns
PARP1 Chemiluminescent Assay Kit	80551	96 rxns
PARP2 Chemiluminescent Assay Kit	80552	96 rxns.
PARP3 Chemiluminescent Assay Kit	80553	96 rxns.
PARP5A (TNKS1) Chemiluminescent Assay Kit	80573	96 rxns.
PARP5B (TNKS2) Chemiluminescent Assay Kit	80579	96 rxns.
PARP6 Chemiluminescent 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/C) Enzyme	80505	10 µg
PARP7 Enzyme	80507	10 µg
PARP9 Enzyme	80509	10 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Colorimetric signal of positive control reaction is weak	Enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity.
	Colorimetric HRP substrate was not incubated long enough	Increase the amount of time that the colorimetric HRP substrate is incubated in the wells. Avoid azides.
Colorimetric signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Signal is out of range of detection (too high)	Decrease the amount of time that the colorimetric HRP substrate is incubated in the wells
Background (signal to noise ratio) is high	Insufficient washes or blocking	Be sure to include blocking steps after wash steps. Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST. Be sure to dilute Streptavidin-HRP in blocking buffer, not assay buffer.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1%. Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of enzyme to create a standard curve.

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