6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

TNKS2 Histone Ribosylation Assay Kit (Antibody Detection)

Catalog # 80576

DESCRIPTION: The *TNKS2 Histone Ribosylation Assay Kit (Antibody Detection)* is designed to measure Tankyrase-2 activity for screening and profiling applications. The *TNKS2 Histone Ribosylation Assay Kit (Antibody Detection)* comes in a convenient format, with 8-well strips, histone mixture substrate, antibody against poly (ADP-ribose) modified histone, the secondary HRP-labeled antibody, NAD+, PARP assay buffer, and purified TNKS2 enzyme for 100 enzyme reactions. The key to the *TNKS2 Histone Ribosylation Assay Kit (Antibody Detection)* is a highly specific antibody that recognizes poly (ADP-ribose)-modified histone. After coating the plate with the histone mixture, only three simple steps are required. First, NAD+ is incubated with a sample containing assay buffer and enzyme for one hour. Next, primary antibody is added. Finally, the plate is treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.
Note: This kit is also suitable for use with cell extracts, but non-specific ribosylation activity may be detected.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
80515	TNKS2	2 µg	-80°C	***(Avoid freeze/thaw cycles!)***
	5× histone mixture	1 ml	-80°C	
	NAD+	250 µl	-80°C	
	10x PARP Assay Buffer	1 ml	-20°C	
52140K	Primary antibody 11	13 µl	-80°C	
52130H	Secondary HRP-labeled antibody 1	10 µl	-80°C	
	Blocking Buffer	80 ml	+4°C	
	HRP chemiluminescent substrate (2 components)	6 ml each	+4°C	
	8-well strip plate module	1 plate (12 x 8-well strips)_	+4°C	

MATERIALS REQUIRED BUT NOT SUPPLIED:
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
Paper towels

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

CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt

STABILITY: 6 months from date of receipt when stored as directed.

REFERENCE: Dillon SC, Zhang X, Trievel RC, Cheng X. *Genome Biology* 2005; **6:**227.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Coating the plate with the histone mixture:

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

2) Add 50 µl diluted histone mixture to each well and incubate for 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 30 min at room temperature.

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

Step 1:

1) Prepare the master mixture: N wells x (5 µl **10x PARP assay buffer** + 2.5 µl **NAD+** +17.5 µl **H_2O**)

2) Thaw **TNKS2 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **TNKS2 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: TNKS2 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute **TNKS2 enzyme** in 1X PARP assay buffer at 1 ng/µl (20 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

4) Add 25 µl of master mixture to each well designated for the “Positive Control”, “Test Inhibitor”, and “Blank”. For the “Substrate Control”, add 5 µl **10x PARP assay buffer** +20 µl **H_2O.**

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	Positive Control	Test Inhibitor	Substrate Control	Blank
10X PARP Assay Buffer	5 µl	5 µl	5 µl	5 µl
NAD+	2.5 µl	2.5 µl	–	2.5 µl
H_2O	17.5 µl	17.5 µl	20 µl	17.5 µl
Test Inhibitor	–	5 µl	–	–
Inhibitor buffer (no inhibitor)	5 µl		5 µl	5 µl
1 x PARP buffer	-	-	-	20 µl
TNKS2 (1 ng/µl)	20 µl	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl	50 µl

5) 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).

6) Add 20 µl of 1 x PARP buffer to the well designated “Blank”.

7) Initiate TNKS reactions by adding 20 µl of diluted TNKS2 prepared above. Incubate the reactions for 1 hour at room temperature.

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

9) Add 100 µl of Blocking buffer to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

Step 2:

1) Dilute **Primary antibody 11** 800-fold with Blocking buffer.
2) Add 150 µl per well. Incubate 1 hour at room temperature with slow shaking.
3) Wash strip plate with PBST buffer and Blocking buffer as in steps 1-8 and 1-9.

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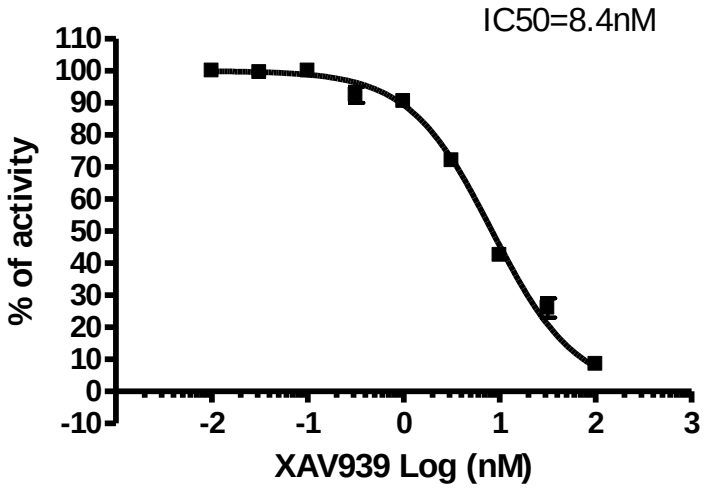
Step 3:

1) Dilute **Secondary HRP-labeled antibody 1** 1,000-fold with Blocking buffer.
2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.
3) Wash strip plate with PBST buffer and Blocking buffer as in steps 1-8 and 1-9.
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.
5) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

Example of Assay Results:



Inhibition of TNKS2 by XAV939, measured using the *TNKS2 Histone Ribosylation Assay Kit (Antibody Detection)*, BPS Bioscience #80576. 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 Name	Catalog #	Size
TNKS1 Histone Ribosylation Assay Kit (Biotin-labeled NAD+)	80574	96 rxns.
PARP1 Chemiluminescent Assay Kit	80551	96 rxns.
PARP2 Chemiluminescent Assay Kit	80552	96 rxns.
PARP3 Chemiluminescent Assay Kit	80553	96 rxns.
PARP6 Chemiluminescent Assay Kit	80556	32 rxns.
PARP7 Chemiluminescent Assay Kit	80557	96 rxns.
PARP11 Chemiluminescent Assay Kit	80561	96 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

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	TNKS2 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (TNKS2, BPS Bioscience #80515). Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Record light signals at 5 second intervals. Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent 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.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in PBST.
	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 (TNKS2, BPS Bioscience #80515) to create a standard curve.