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Data Sheet

TNKS1 Histone Ribosylation Assay Kit (Antibody Detection)

Catalog # 80575
Size: 384 reactions

DESCRIPTION: The TNKS1 Histone Ribosylation Assay Kit (Antibody Detection) is designed to measure Tankyrase-1 activity using purified Tankyrase-1 (TNKS1) or cell extracts containing TNKS1. The TNKS1 Histone Ribosylation Assay Kit (Antibody Detection) comes in a convenient format, with a 384-well plate precoated with histone mixture, antibody against poly (ADP-ribose) modified histone, the secondary HRP-labeled antibody, NAD+, PARP assay buffer, and purified TNKS1 enzyme for 384 enzyme reactions. The key to the TNKS1 Histone Ribosylation Assay Kit (Antibody Detection) is a highly specific antibody that recognizes poly (ADP-ribose)-modified histone. With this kit, only three simple steps on a microtiter plate are required for Tankyrase-1 reaction. 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	
80504	TNKS1	4 µg	-80°C	***(Avoid freeze/thaw cycles!)***
	NAD+	500 µl	-80°C	
	10x PARP Assay Buffer	2 ml	-20°C	
52140K	Primary antibody 11	26 µl	-80°C	
52130H	Secondary HRP-labeled antibody 1	20 µl	-80°C	
	Blocking Buffer	160 ml	+4°C	
	HRP chemiluminescent substrate (2 components)	12 ml each	+4°C	
	384-well plate pre-coated with histone mixture	1 plate	+4°C	

MATERIALS REQUIRED BUT NOT SUPPLIED:
PBST buffer (1x PBS, containing 0.05% Tween20)
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.

Step 1:

1) Rehydrate the microwells by adding 50 µl of PBST buffer (1 x PBS, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the strip plate onto clean paper towels to remove liquid.

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. *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 ng/µl (10 ng/10 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

4) Using master mixes as much as possible, add the following reagents to the microwells, in duplicate:

	Positive Control	**Test Sample**	**Substrate Control**	**Blank**
TNKS1 (1 ng/µl)	10 µl	10 µl	10 µl	–
10X PARP Assay Buffer	2.5 µl	2.5 µl	2.5 µl	2.5 µl
NAD+	1.25 µl	1.25 µl	–	1.25 µl
Test Inhibitor/Activator	–	X µl	–	–
H_2O	11.25 µl	11.25 – X µl	12.5 µl	21.25 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

5) Add the entire reaction mixture (25 µl) to the substrate-coated plate. Incubate at room temperature for 1 hour.

6) Wash the plate three times with PBST buffer. Blot dry onto clean paper towels.

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7) Add 50 µl of Blocking buffer to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

Step 2:

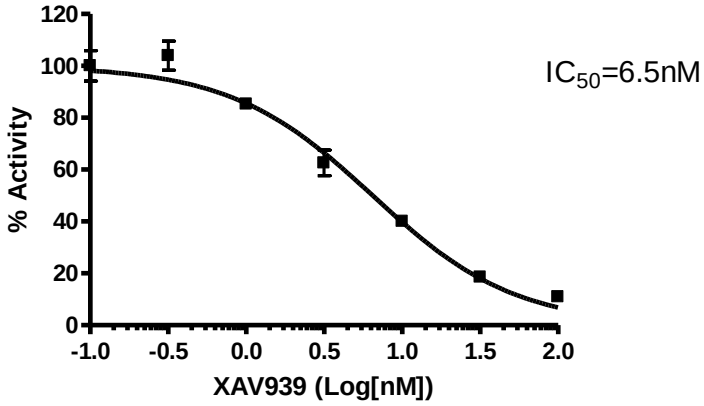
1) Dilute "Primary antibody 11" 800-fold with Blocking buffer.
2) Add 50 µl per well. Incubate 1 hour at room temperature with slow shaking.
3) Wash plate with PBST buffer and Blocking buffer as in steps 1-6 and 1-7.

Step 3:

1) Dilute "Secondary HRP-labeled antibody 1" 1,000-fold with Blocking buffer.
2) Add 50 µl per well. Incubate for 30 min. at room temperature with slow shaking.
3) Wash plate with PBST buffer and Blocking buffer as in steps 1-6 and 1-7.
4) Just before use, mix on ice 25 µl HRP chemiluminescent substrate A and 25 µl HRP chemiluminescent substrate B and add 50 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:

TNKS1 + XAV939



Inhibition of TNKS1 by XAV939, measured using the TNKS1 Histone Ribosylation Assay Kit (Antibody Detection), BPS Bioscience # 80574/80575. 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
TNKS2 Histone Ribosylation Assay Kit	80577	384 rxns.
TNKS1 Histone Ribosylation Assay Kit (Biotin-labeled NAD+)	80573	96 rxns.
TNKS1 Histone Ribosylation Assay Kit (Antibody Detection	80574	96 rxns.
TNKS2 Histone Ribosylation Assay Kit	80576	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	TNKS1 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (TNKS1, BPS Bioscience #80504). 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 (TNKS1, BPS Bioscience #80504) to create a standard curve.