Description

The TNKS1 Colorimetric Assay Kit is designed to measure the activity of Tankyrase 1 (TNKS1, also known as PARP5A) for screening and profiling applications. TNKS1 is known to catalyze the NAD-dependent ADP-ribosylation of histones. 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 (PARP5A) Colorimetric Assay Kit is the biotinylated NAD+. First, histone proteins are coated on a 96-well plate. Next, a biotinylated NAD+ mix (termed PARP Substrate Mixture) is incubated with the TNKS1 enzyme. Finally, the plate is treated with streptavidin-HRP followed by addition of the colorimetric HRP substrate to produce color that can be measured using a UV/Vis spectrophotometer microplate reader.

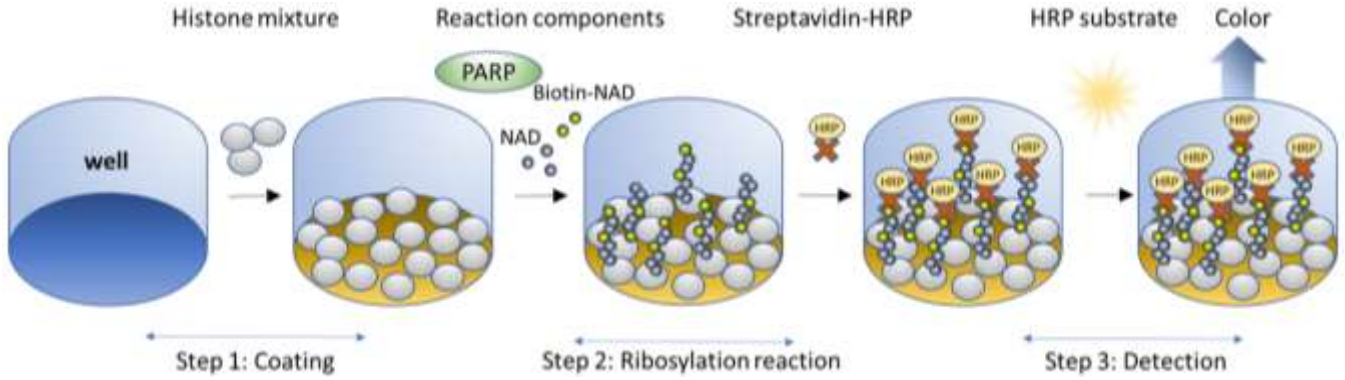

Figure 1. TNKS1 (PARP5A) Colorimetric Assay Kit schematic

**NOTE: As of March 2023, this protocol has been re-optimized for performance. Previous versions of this kit are available upon request.*

Applications

Study enzyme kinetics and screen small molecular inhibitors for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage	
80504	TNKS1* (Tankyrase 1 (PARP5A), GST-Tag)	3 µg	-80°C	***Avoid multiple freeze/ thaw cycles***
52029	5x Histone Mixture	1 ml	-80°C	
78371	PARP Substrate Mixture 2	4 x 250 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	
79651	Colorimetric HRP substrate	10 ml	+4°C	
79964	96-well clear plate		Room Temp	

**The concentration of the protein is lot-specific and will be indicated on the tube. Excess material has been given for ease of retrieval.*

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Materials Required but Not Supplied

- DTT (10 mM in water, prepared fresh
- 1x PBS (phosphate buffer saline)
- PBST: 1x PBS, containing 0.05% Tween-20
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*
- 2 M sulfuric acid (aqueous)
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

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

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/thaw cycles!***

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The TNKS1 (PARP5A) Colorimetric Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in no higher than 10% DMSO solution and using 5 µl per well.

Assay Protocol

- All samples and controls should be performed in duplicates.
- The assay should include a "Blank" and a "Positive control."

Step 1: Coat 50 µl of histone solution to a 96-well module (VWR catalog no. 62409-300)

1) Dilute 5x histone mixture 1:5 with PBS to make 1x histone mixture.

2) Add 50 µl of histone mixture to each well and incubate at 4°C overnight.

3) Wash the plate three times using 200 µl of PBST (1x PBS containing 0.05% Tween 20) per well.

4) Tap the plate onto a clean paper towel to remove the liquid.

5) Block the wells by adding 200 µl of Blocking buffer 3 to every well. Incubate at room temperature for at least 90 minutes.

6) Wash plate three times with 200 µl of PBST.

7) Tap the plate onto clean paper towel to remove the liquid.

Step 2: Ribosylation reaction

1) Prepare a fresh solution of 10 mM DTT in water

2) Prepare the Master Mix (25 µl/well): N wells (2.5 µl of 10x PARP buffer + 10 µl of PARP Substrate Mixture 2 + 10 µl of water + 2.5 µl of 10 mM fresh DTT).

3) Add 25 µl of Master Mix to every well.

4) Prepare 1x PARP assay buffer with DTT. Dilute 10x PARP assay buffer to 1x PARP assay buffer containing DTT by adding 1 volume of 10x PARP assay buffer + 1 volume of 10 mM DTT + 8 volumes of distilled water.

Note: the concentration of DTT in the 1x PARP assay buffer will be 1 mM

5) Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.
   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the PARP assay buffer, at concentrations 10-fold higher than the desired final concentrations. For the positive and negative controls, use 1x PARP assay buffer containing DTT (Diluent Solution).
   OR
   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in PARP assay buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.
   Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in PARP assay buffer to keep the concentration of DMSO constant. For positive and negative controls, prepare 10% DMSO in PARP assay buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).
   Note: The TNKS1 (PARP5A) Colorimetric Assay Kit is compatible with up to 1% final DMSO concentration

6) Add 5 µl of Test Inhibitor to each well labeled as "Test Inhibitor."
For the "Positive Control" and "Blank," add 5 µl of the same diluent solution used to dilute the inhibitor, but without inhibitor (Diluent Solution).

7) Thaw **TNKS1 enzyme** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Prepare enough TNKS1 for this portion of the assay and aliquot the remaining undiluted protein into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C. Do not re-use these aliquots more than once.

   Dilute the enzyme to **1.5 ng/µl** with 1x PARP assay buffer. The final concentration of TNKS1 will be 10 nM.

   Note: TNKS1 enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. ***Do not re-use the diluted enzyme.***

8) Initiate the reaction by adding 20 µl of diluted TNKS1 enzyme to the wells designated “Positive Control” and "Test Inhibitor."

   To the wells designated as "Blank," add 20 µl of 1x PARP assay buffer.

9) Incubate at room temperature for 1 hour.

	Blank	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x PARP assay buffer	20 µl	-	-
TNKS1 (1.5 ng/µl)		20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

10) Wash the plate three times with 200 µl of PBST and tap the plate onto a clean paper towel.

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 of PBST and tap the plate onto a clean paper towel.

4) Add 100 µl of the colorimetric HRP substrate to each well and incubate the plate at the room temperature until a blue color has 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.

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. 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 Results

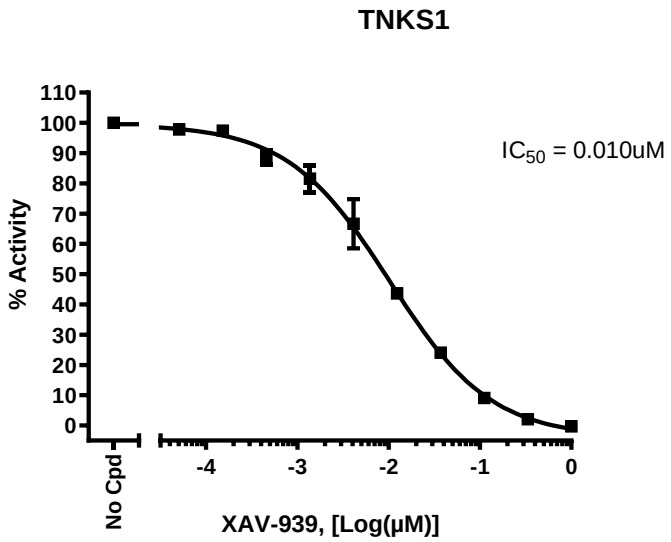

Figure 1: TNKS1 (PARP5A) activity in the presence of increasing concentrations of XAV939.
The effect of XAV939 (Cayman Chemical #13596) was measured using the TNKS1 (PARP5A) Colorimetric Assay Kit (BPS Bioscience #78576). Luminescence was measured using a Bio-Tek microplate reader.

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
PARP1 Chemiluminescent Assay Kit	80551	96 reactions
PARP1 Colorimetric Assay Kit	80580	96 reactions
PARP2 Chemiluminescent Assay Kit	80552	96 reactions
PARP2 Colorimetric Assay Kit	80581	96 reactions
PARP3 Chemiluminescent Assay Kit	80553	96 reactions
PARP6 Chemiluminescent Assay Kit	80556	96 reactions
PARP7 Chemiluminescent Assay Kit	79729	96 reactions
PARP10 Chemiluminescent Assay Kit	80560	96 reactions
PARP11 Chemiluminescent Assay Kit	80561	96 reactions
PARP14 Chemiluminescent Assay Kit	80568	96 reactions
PARP15 Chemiluminescent Assay Kit	80567	96 reactions
TNKS2 (PARP5B) Chemiluminescent Assay Kit	78406	96 reactions
PARP1, GST-tag	80501	20 µg
PARP2, GST-tag	80502	10 µg
PARP3, GST-tag	80503	10 µg
PARP6, GST-tag	80506	10 µg
PARP7, FLAG-tag	80527	10 µg
PARP10, FLAG-Strep-Tag	80522	10 µg
PARP11, GST-Tag, His-Tag	80511	10 µg
PARP12, His-GST-tag	80513	10 µg
PARP14, His-GST-Tag	80514	10 µg
PARP15, GST-tag	80517	10 µg
Tankyrase 1 (PARP5A), GST-tag	80504	10 µg
Tankyrase 2 (PARP5B) [849-1166], GST-tag	80515	10 µg
PARPtrap™ Assay Kit for PARP1	80584	2 sizes
PARPtrap™ Assay Kit for PARP2	78296	2 sizes