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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 |
| | Blocking buffer | 25 ml | +4°C |
| | Streptavidin-HRP | 100 µl | +4°C |
| | Colorimetric HRP substrate | 10 ml | +4°C |
| | 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.

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

**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 three 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 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 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 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**.

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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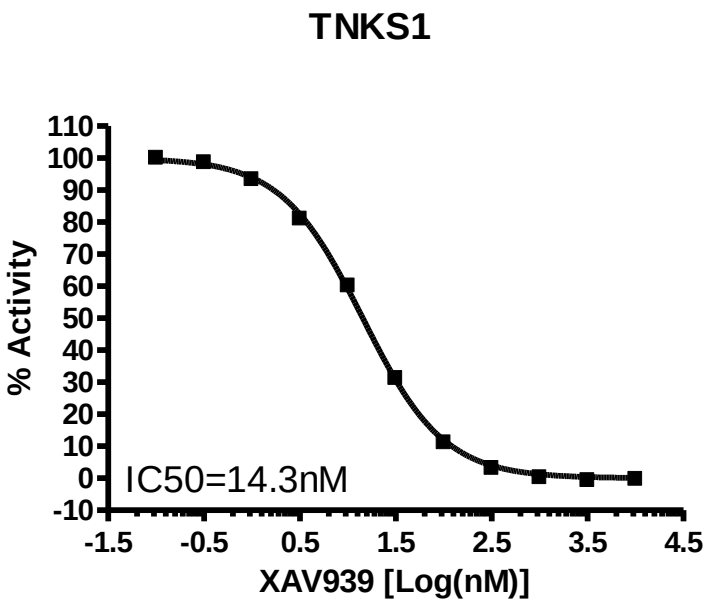
5) After the blue color is developed, add 100 µl of 2 M sulfuric acid to each well. Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader. The blank wells should exhibit an absorbance of ~ 0.05 at 450 nm. *Alternatively, the plate may be read at 650 nm without adding 2 M sulfuric 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.<br>Increase time of enzyme incubation.<br>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.<br>Use a multichannel pipettor.<br>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.<br>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%.<br>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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