**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+. With this kit, only three simple steps are required for TNKS1 reactions. 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 in an optimized assay buffer. 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.

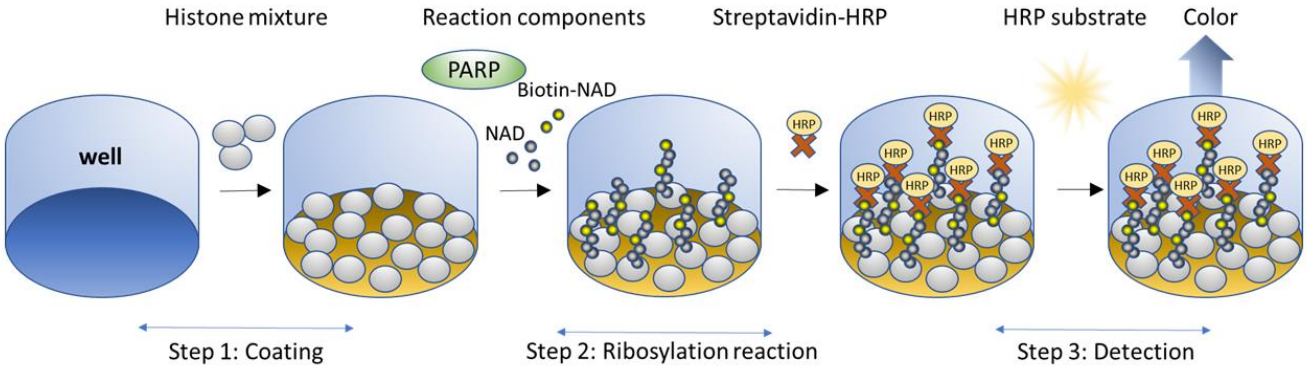


**Figure 1.** TNKS1 (PARP5A) Colorimetric Assay Kit schematic

**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) | 1 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 transparent 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.*

**Materials Required but Not Supplied**

- 1x PBS (phosphate buffer saline) buffer
- PBST buffer (1x PBS, containing 0.05% Tween-20)
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*
- 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 transparent plate**

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 PBST buffer (1x PBS containing 0.05% Tween 20) per well.

4) Tap the plate onto 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 PBST buffer.

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

**Step 2: Ribosylation reaction**

1) Prepare the Master Mix (25 µl/well): N wells x (2.5 µl of 10x PARP buffer + 10 µl of PARP Substrate Mixture 2 + 12.5 µl of water).

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

3) Prepare 1x PARP buffer. Dilute 10x PARP assay buffer to 1x PARP assay buffer by adding 1 volume of 10x PARP assay buffer + 9 volumes of water.

4) 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).

   *Note: The TNKS1 (PARP5A) Colorimetric Assay Kit is compatible with up to 1% final DMSO concentration. If the inhibitor is dissolved in DMSO, we recommend preparing the inhibitor in 10% DMSO aqueous solution and using 5 µl per reaction.*

   For example, if the compound is dissolved in DMSO, make a 100-fold higher concentration of the compound in DMSO than the highest concentration you want to test in the assay. Then dilute 10-fold in 1x PARP buffer. At this step the compound concentration is 10-fold higher than the desired final concentration. If you want to run an $IC_{50}$ or test lower concentrations of the compound, prepare serial dilutions using 1x PARP buffer containing 10% DMSO, so the final concentration of DMSO will remain at 1% final.

   If the compound is soluble in water, use the 1x PARP assay buffer to prepare the test inhibitor.

5) Thaw TNKS1 enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Calculate the amount of TNKS1 required for the assay and dilute enzyme to **0.15 ng/µl** with 1x PARP buffer. The final concentration of TNKS1 will be 1 nM. Aliquot the remaining undiluted TNKS1 enzyme into aliquots and store at -80°C. Do not re-use these aliquots more than once.

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

6) 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 buffer.

   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 buffer with DTT | 20 µl | - | - |
| TNKS1 (0.15 ng/µl) | - | 20 µl | 20 µl |
| **Total** | **50 µl** | **50 µl** | **50 µl** |

7) Wash the plate three times with 200 µl PBST buffer and tap the plate onto 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 PBST buffer and tap the plate onto clean paper towel.

4) Add 100 µl of the Colorimetric HRP Substrate to each well and incubate the plate at room temperature until blue color is developed in the positive control well. For TNKS1, it normally takes 15-20 minutes 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. 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 Results**

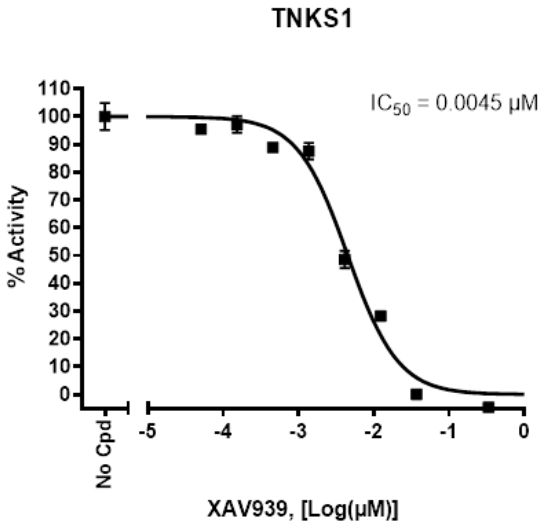


*Figure 1: TNKS1 (PARP5A) activity in the presence of increasing concentrations of XAV939.* *The effect of XAV939* (Cayman Chemical #13596) was measured using the TNKS1 (PARP5A) Colorimetric Assay Kit (BPS Bioscience #80582). Absorbance at 450 nm was measured using a Tecan microplate reader.

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 |
| PARP2 Chemiluminescent Assay Kit | 80552 | 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 |