



6042 Cornerstone Court W, Ste B  
San Diego, CA 92121  
Tel: 1.858.202.1401  
Fax: 1.858.481.8694  
Email: info@bpsbioscience.com

## Data Sheet

### ***TNKS2 Histone Ribosylation Assay Kit (Biotin-labeled NAD<sup>+</sup>)*** **Catalog # 80578**

**DESCRIPTION:** The TNKS2 Histone Ribosylation Assay Kit (Biotin-labeled NAD<sup>+</sup>) is designed to measure Tankyrase 2 (TNKS2) activity for screening and profiling applications. TNKS2 catalyzes the NAD-dependent addition of poly(ADP-ribose) to the substrate proteins. The TNKS2 assay kit comes in a convenient 96-well format, with purified TNKS2 enzyme, histone mixture, and PARP assay buffer for 100 enzyme reactions. The key to the TNKS2 Histone Ribosylation Assay is the biotinylated NAD<sup>+</sup> substrate. With this kit, only three simple steps are required for TNKS2 reactions. First, histone proteins are coated on a 96-well plate. Next, the biotinylated NAD<sup>+</sup> substrate is incubated with an assay buffer that contains the TNKS2 enzyme. Finally, the plate is treated with streptavidin-HRP followed by addition of the HRP substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.

#### **COMPONENTS:**

Catalog #	Component	Amount	Storage
80515	TNKS2	2 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
79743	Blocking buffer 3	25 ml	+4°C
80611	Streptavidin-HRP	100 µl	+4°C
79670	ELISA ECL substrate (2 components)	6 ml each	Room Temperature
79837	96-well module plate	1	Room Temperature

#### **MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

1x 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

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

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)



6042 Cornerstone Court W, Ste B  
San Diego, CA 92121  
Tel: 1.858.202.1401  
Fax: 1.858.481.8694  
Email: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

**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  $\mu$ l diluted histone mixture to each well and incubate overnight at 4°C.
- 3) Wash the plate 3 times with 200  $\mu$ l PBST buffer.
- 4) Block the wells by adding 150  $\mu$ l of Blocking buffer to every well. Incubate for 60 minutes at room temperature.
- 5) Wash the plate 3 times with 200  $\mu$ l 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  $\mu$ l **10x PARP assay buffer** + 2.5  $\mu$ l **10x PARP assay mixture** + 20  $\mu$ l **H<sub>2</sub>O**)
- 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 0.75 – 1 ng/ $\mu$ l (15 - 20 ng/20  $\mu$ l). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
- 4) Add 25  $\mu$ l of master mixture to each well designated for the “Positive Control”, “Test Inhibitor”, and “Blank”. For the “Substrate Control”, add 2.5  $\mu$ l **10x PARP assay buffer** + 22.5  $\mu$ l **H<sub>2</sub>O**.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)



6042 Cornerstone Court W, Ste B  
San Diego, CA 92121  
Tel: 1.858.202.1401  
Fax: 1.858.481.8694  
Email: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

	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
H <sub>2</sub> O	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	-	-	-
TNKS2 (~ 0.75 ng/µl)	-	20 µl	20 µl	20 µl
<b>Total</b>	<b>50 µl</b>	<b>50 µl</b>	<b>50 µl</b>	<b>50 µl</b>

- 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 1x PARP buffer to the well designated "Blank".
- 7) Initiate the reactions by adding 20 µl of diluted TNKS2 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) Just before use, mix on ice 50 µl **ELISA ECL substrate A** and 50 µl **ELISA ECL 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.

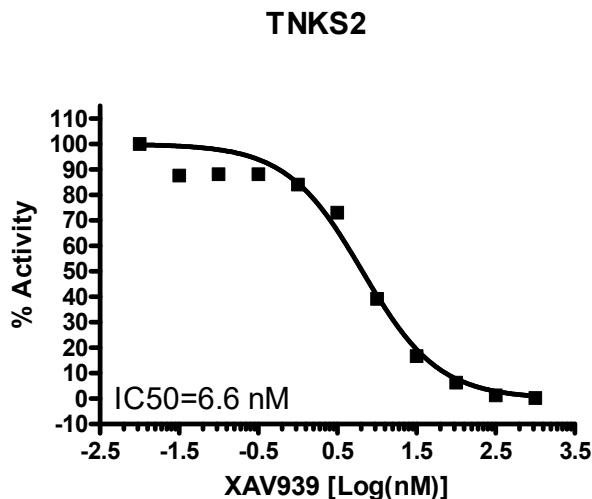
OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)

**Example of Assay Results:**



Inhibition of TNKS2 enzyme (BPS Bioscience, #80515) with XAV939 (BPS Bioscience, #27100), measured using the *TNKS2 Histone Ribosylation Assay Kit (Biotin-labeled NAD<sup>+</sup>)*, BPS Bioscience (Catalog # 80578). 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](mailto:info@bpsbioscience.com)*

**RELATED PRODUCTS:**

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
PARP2 Assay Kit	#80552	96 rxns.
PARP3 Assay Kit	#80553	96 rxns.
TNKS1 Histone Ribosylation Assay Kit (Antibody Detection)	#80574	96 rxns.
PARP5b (TNKS2) Assay Kit	#80576	96 rxns.
PARP6 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), (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

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)