

TREVIGEN[®] Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

Tankyrase 1 Chemiluminescent Activity Assay

96 Tests

Cat# 4701-096-K

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

Chemiluminescent assay kit for candidate inhibitor screening and determination of IC₅₀ values of Tankyrase 1 inhibitors.

Trevigen, Inc.

8405 Helgerman Ct. Gaithersburg, MD 20877

Tel: 1-800-873-8443 • 301-216-2800

Fax: 301-560-4973

e-mail: info@trevigen.com

www.trevigen.com



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VIII. Troubleshooting

PROBLEM	CAUSE	SOLUTION
No light output in wells with Tankyrase 1 alone	Active Tankyrase 1 enzyme was not added.	Add Tankyrase 1 or order fresh Tankyrase 1 (cat# 4700-096-01) and add serial dilutions of Tankyrase 1 in triplicate.
High background in wells with no Tankyrase 1	Poor washing	Increase the number of washes with 1X PBS + 0.1% Triton [®] X-100 after the ribosylation reaction and after incubation with antibodies. Two washes with 1X PBS must follow washes with 1X PBS + 0.1% Triton [®] X-100.

IX. References

1. Hsiao, S.J., Smith, S., Tankyrase function at telomeres, spindle poles, and beyond. *Biochimie*, 2008. **90**:83-92.
2. Reya, T. and H. Clevers. 2005. Wnt signaling in stem cells and cancer. *Nature* **434**:843-50.
3. Huang, S-M. A., Y.M. Mishina, S. Liu, A. Cheung, F. Stegmeier, et al. 2009. Tankyrase inhibition stabilizes axin and antagonizes Wnt signaling. *Nature* **461**:614-20.
4. Dodge, M.E. and Lum, L. 2011. Drugging the cancer stem cell compartment: Lessons learned from the hedgehog and Wnt signal transduction pathways. *Annu. Rev. Pharmacol. Toxicol.* **51**:289-310.

X. Related Products Available From Trevigen

Catalog #	Description	Size
4700-096-01	Tankyrase 1, 10 mUnits/μl	100 μl
4684-096-P	Histone-Coated Natural Strip Well Plates, I-PAR	1 plate
4700-096-K	Tankyrase 1 Colorimetric Activity Assay	96 Tests
4690-096-K	HT F Homogeneous PARP Inhibition Assay Kit	96 tests
4520-096-K	PARP in vivo Pharmacodynamic Assay II	96 tests
4676-096-K	HT Universal Chemiluminescent PARP Assay Kit/w Histone-Coated Strip Wells	96 samples
4677-096-K	HT Universal Colorimetric PARP Assay Kit/w Histone-Coated Strip Wells	96 samples
4684-096-K	HT Colorimetric PARP Apoptosis Assay	96 tests
4685-096-K	HT Chemiluminescent PARP Apoptosis Assay	96 tests
4667-250-01	Recombinant Human PARP Enzyme	250 μl
4668-100-1	Recombinant Human PARP (High Specific Activity)	1000 Units
4682-096-K	HT Chemiluminescent PARG Assay Kit	96 tests
4683-096-K	HT Colorimetric PARG Assay Kit	96 tests

Note: Tankyrase Standard Curve utilizes 25 μ l of each Tankyrase dilution. The XAV939 Inhibition Curve combines 25 μ l of 5 mU/well Tankyrase with 25 μ l of inhibitor assay cocktail. Using a reagent plate to make enzyme dilutions and dispensing with a multichannel pipet is recommended to minimize the variability in data as a consequence of the time required to add enzyme to each well. **Diluted enzyme should be used immediately and any remainder discarded.**

7. Antibody Diluent

This solution is used as a diluent for the antibodies. Dilute the 5X Antibody Diluent (cat# 4684-096-03) 1:5 with dH₂O before use.

8. Anti-PAR Monoclonal Antibody

Just before use, dilute the anti-PAR monoclonal antibody (cat# 4684-096-04) 1,000-fold with 1X Antibody Diluent (cat# 4684-096-03). A total of 50 μ l/well of diluted anti-PAR monoclonal antibody is required in the assay.

9. Goat Anti-Mouse-IgG-HRP Conjugate

Just before use, dilute the goat anti-mouse IgG-HRP conjugate (cat# 4684-096-05) 1,000-fold with 1X Antibody Diluent (cat# 4684-096-03). A total of 50 μ l/well of diluted goat anti-mouse IgG-HRP conjugate is required in the assay.

10. PARP PeroxyGlow™ A and B

Prewarm PARP PeroxyGlow A and B to room temperature before use. Just before use, mix equal volumes of PARP PeroxyGlow™ A and B together and add 100 μ l per well. PARP PeroxyGlow™ A and B are Horseradish Peroxidase (HRP) substrates generating light that can be quantified in a suitable chemiluminescent plate reader.

11. Histone-Coated White Strip Well Plates, I-PAR

The white strip wells are precoated with histones that are ribosylated by Tankyrase 1. Unused stripwells should be immediately removed from the plate and stored at 4 °C under desiccation for future use. Figure 1 provides an example of how the plate may be configured to evaluate the half maximal inhibitory concentration (IC₅₀) of a Tankyrase 1 inhibitor, such as XAV939.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10 mU Tankyrase 1			500 nM XAV939								
B	5 mU Tankyrase 1			100 nM XAV939								
C	2.5 mU Tankyrase 1			50 nM XAV939								
D	1 mU Tankyrase 1			10 nM XAV939								
E	0.5 mU Tankyrase 1			5 nM XAV939								
F	0.25 mU Tankyrase 1			1 nM XAV939								
G	0.1 mU Tankyrase 1			0.5 nM XAV939								
H	0 mU Tankyrase 1			0 nM XAV939								

Figure 1. Sample Plate Setup

Tankyrase 1 Chemiluminescent Activity Assay

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I. Introduction

Trevigen's **Tankyrase 1 Chemiluminescent Activity Assay** is a highly sensitive chemiluminescent screening kit for the identification of Tankyrase 1 inhibitors in an *in vitro* system. Tankyrase 1, also known as PARP5a, is a telomere associated poly (ADP-ribose) polymerase that is recruited to telomeres via its interaction with TRF1 and plays a role in the maintenance of telomere length, sister telomere association, and mitotic spindle organization¹. Changes in telomere function have been associated with various diseases, including cancer and aging. Tankyrase 1 has also been shown to destabilize axin, thereby activating Wnt signaling and driving tumor cell proliferation^{2,3}. Given these known activities, Tankyrase 1 has become a prominent cancer drug target⁴.

Trevigen's **Tankyrase 1 Chemiluminescent Activity Assay** is ideal for the screening of Tankyrase 1 inhibitors and determining IC₅₀ values. The **Tankyrase 1 Chemiluminescent Activity Assay** is an ELISA which semi-quantitatively detects poly (ADP-ribose) (PAR) deposited onto immobilized histone proteins in a 96-well format. An anti-PAR monoclonal antibody, goat anti-mouse IgG-HRP conjugate, and HRP substrate are used to generate a chemiluminescent signal. Thus, light output correlates with Tankyrase 1 activity. Important features of the assay include: 1) chemiluminescent, non-radioactive format; 2) higher throughput 96 test size, and 3) sensitivity down to 0.1 mUnits of Tankyrase 1.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of the chemicals and reagents contained in the Tankyrase 1 Chemiluminescent Activity Assay may not yet have been fully investigated. Therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using any of these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products. MSDS are available on request.

III. Materials Supplied

Catalog #	Component	Amount	Storage
4684-096-03	5X Antibody Diluent	3 ml	4 °C
4684-096-04	Anti-PAR monoclonal antibody	20 µl	-20 °C
4684-096-05	Goat anti-mouse IgG-HRP	20 µl	-20 °C
4684-096-07	20X I-PAR Assay Buffer	2.5 ml	-20 °C
4685-096-P	Histone-Coated White Strip Well Plates, I-PAR	96 wells	4 °C
4700-096-01	Tankyrase 1, 10 mUnits/µl	100 µl	-20 °C
4700-096-02	Assay Substrate	2 ml	-20 °C
4700-096-03	XAV939, 1 mM	20 µl	-20 °C
4675-096-01	PARP PeroxyGlow™ A	6 ml	4 °C
4675-096-02	PARP PeroxyGlow™ B	6 ml	4 °C

IV. Materials/Equipment Required But Not Supplied

Reagents:

1. Inhibitors
2. 10X PBS (cat# 4870-500-6)
3. PBS + 0.1% Triton® X-100
4. Distilled water

Note: Tankyrase Standard Curve utilizes 25 µl of each Tankyrase dilution. The XAV939 Inhibition Curve combines 25 µl of 5 mU/well Tankyrase with 25 µl of inhibitor assay cocktail. Using a reagent plate to make enzyme dilutions and dispensing with a multichannel pipet is recommended to minimize the variability in data as a consequence of the time required to add enzyme to each well. **Diluted enzyme should be used immediately and any remainder discarded.**

7. Antibody Diluent

This solution is used as a diluent for the antibodies. Dilute the 5X Antibody Diluent (cat# 4684-096-03) **1:5** with dH₂O before use.

8. Anti-PAR Monoclonal Antibody

Just before use, dilute the anti-PAR monoclonal antibody (cat# 4684-096-04) 1,000-fold with **1X** Antibody Diluent (cat# 4684-096-03). A total of 50 µl/well of diluted anti-PAR monoclonal antibody is required in the assay.

9. Goat Anti-Mouse-IgG-HRP Conjugate

Just before use, dilute the goat anti-mouse IgG-HRP conjugate (cat# 4684-096-05) 1,000-fold with **1X** Antibody Diluent (cat# 4684-096-03). A total of 50 µl/well of diluted goat anti-mouse IgG-HRP conjugate is required in the assay.

10. PARP PeroxyGlow™ A and B

Prewarm PARP PeroxyGlow A and B to room temperature before use. Just before use, mix equal volumes of PARP PeroxyGlow™ A and B together and add 100 µl per well. PARP PeroxyGlow™ A and B are Horseradish Peroxidase (HRP) substrates generating light that can be quantified in a suitable chemiluminescent plate reader.

11. Histone-Coated White Strip Well Plates, I-PAR

The white strip wells are precoated with histones that are ribosylated by Tankyrase 1. Unused stripwells should be immediately removed from the plate and stored at 4 °C under desiccation for future use. Figure 1 provides an example of how the plate may be configured to evaluate the half maximal inhibitory concentration (IC₅₀) of a Tankyrase 1 inhibitor, such as XAV939.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10 mU Tankyrase 1		500 nM XAV939									
B	5 mU Tankyrase 1		100 nM XAV939									
C	2.5 mU Tankyrase 1		50 nM XAV939									
D	1 mU Tankyrase 1		10 nM XAV939									
E	0.5 mU Tankyrase 1		5 nM XAV939									
F	0.25 mU Tankyrase 1		1 nM XAV939									
G	0.1 mU Tankyrase 1		0.5 nM XAV939									
H	0 mU Tankyrase 1		0 nM XAV939									

Figure 1. Sample Plate Setup

VI. Tankyrase 1 Inhibitor Assay Protocol

A. Ribosylation Reaction

Note: Do not premix the Tankyrase enzyme and the Assay Cocktail since Tankyrase will autoribosylate in the presence of NAD.

- Determine assay layout and controls:
 - Negative Control: A negative control without Tankyrase 1 should be prepared to determine background light output.
 - Activity Control for Tankyrase 1 Inhibitor Study: 5 mUnits/well Tankyrase without inhibitors. These wells provide the 100% activity reference point.
 - Tankyrase 1 Standard Curve: Serially dilute the Tankyrase 1 enzyme in a cold reagent plate or microtubes with **1X** I-PAR Assay Buffer such that the total activity is 10 mUnits/25 μ l, 5 mUnits/25 μ l, 2.5 mUnits/25 μ l, 1 mUnits/25 μ l, 0.5 mUnits/25 μ l, 0.25 mUnits/25 μ l, and 0.1 mUnits/25 μ l. Add 25 μ l of each standard to triplicate wells (See Figure 1, columns 1-3).
 - Optional XAV939 Inhibition Curve: Serially dilute the XAV939 in a reagent plate or microtubes with Assay Cocktail such that the concentrations are 1000 nM/25 μ l, 200 nM/25 μ l, 100 nM/25 μ l, 20 nM/25 μ l, 10 nM/25 μ l, 2 nM/25 μ l, and 1 nM/25 μ l (See Figure 1, columns 4-6).
- Remove the strip wells from the wrapper, and add 50 μ l/well of 1X I-PAR Assay Buffer to rehydrate the histones. Incubate at 25°C for 30 minutes. Remove the 1X I-PAR Assay Buffer from the wells by tapping the strip wells on paper towels.
- Prepare Tankyrase 1 standard curve assay cocktail (Section **V.4.A**) and inhibitor assay cocktail (Section **V.5**). Add 25 μ l each to the corresponding wells (see Figure 1). The final reaction volume is 50 μ l, so inhibitors should be at two times the desired working concentration, prior to assay.
- Add 25 μ l of Tankyrase 1 enzyme (prepared in Section **V.6.A**) to the wells without inhibitor and 25 μ l of 5 mUnits/well Tankyrase 1 enzyme (prepared in Section **V.6.B**) to the wells containing inhibitor(s), as directed in Figure 1.
- The final reaction volume is 50 μ l:

	<u>Volume</u>	<u>Order of Addition</u>
Assay Cocktail (with or without inhibitor)	25 μ l	1
Tankyrase 1 enzyme	25 μ l	2
Total volume	50 μ l	

Note: The initial inhibitor concentration should be 2-fold that of the final inhibitor concentration in the reaction, since the reaction volume is 50 μ l.

- Incubate the strip wells at 25°C for 30 minutes.

B. Detection

- Wash strip wells 2 times with **1X** PBS + 0.1% Triton[®] X-100 (200 μ l/well) followed by 2 washes with 1X PBS. Ensure that all the liquid is removed following each wash by tapping strip wells onto paper towels.

Equipment:

- Micropipettes and tips
- Multichannel pipettor 10 - 200 μ l
- Incubator set at 25°C
- Wash bottle or microstrip wells washer (optional)
- 96-well chemiluminescent plate reader or luminometer
- Reagent plate (Nunc 262146 or equivalent) or microtubes

V. Reagent Preparation

- Phosphate Buffered Saline (PBS) Wash Solution**
Prepare 500 ml of 1X PBS.
- PBS + 0.1% Triton[®] X-100 Wash Solution**
Prepare 500 ml of 1X PBS containing 0.1% Triton[®] X-100.
- 1X I-PAR Assay Buffer (contains 0.1 mg/ml BSA)**
Dilute the 20X I-PAR Assay Buffer (cat# 4684-096-07) to **1X (1:20)** with dH₂O. The **1X** I-PAR Assay Buffer is used to rehydrate the histone-coated wells and to dilute the enzyme and the inhibitors to be tested.
- Assay Cocktail**
The Assay Cocktail is composed of Assay Substrate (cat# 4700-096-02) and 1X I-PAR Assay Buffer with or without inhibitor for a total volume of 25 μ l per well. This equation may be used to formulate custom reactions:

	<u>Volume (per well)</u>
Assay Substrate	15 μ l
1X I-PAR Assay Buffer (with or without inhibitor)	10 μ l
Total volume	25 μ l

The Assay Cocktail may be prepared in bulk; however, **it must be used immediately and any remainder discarded.**

- For the standard curve, dilute 420 μ l of Assay Substrate with 280 μ l of 1X I-PAR Assay Buffer, and add 25 μ l per well. **Refer to step VI.A.3.**
 - For the inhibitor assay cocktail, dilute 390 μ l of Assay Substrate with 260 μ l of 1X I-PAR Assay Buffer. Use this cocktail to serially dilute a prepared inhibitor stock. **Refer to step V.5.**
- Tankyrase 1 Inhibitors**
XAV939 (Cat# 4700-096-03) is provided at 1 mM in DMSO as a control inhibitor. XAV939 will inhibit the activity of Tankyrase 1 at a wide range of concentrations from 0.5 nM to 500 nM. First prepare 1 μ M XAV939 stock (Table 1), and then serially dilute the stock XAV939 with Assay Cocktail (Section **V.4.B**) to make inhibitor assay cocktails (Table 2). Dilutions may be prepared in a reagent plate and dispensed using a multichannel pipet, or they may be made in individual microtubes and dispensed using a single channel pipet. Add 25 μ l of each inhibitor assay cocktail to designated wells (Figure 1). Other Tankyrase 1 inhibitors may be substituted for XAV939 using the same format. **Refer to step VI.A.3.**

Table 1: XAV939 initial dilution to prepare 1 μM XAV939.

Concentration	24 μM	1 μM
XAV939, 1 mM	5 μl	5 μl
1X I-PAR Assay Buffer	203 μl	43 μl
Assay Substrate	0 μl	72 μl

Table 2: XAV939 serial dilution for sample plate setup.

Conc.	1 μM	200 nM	100 nM	20 nM	10 nM	2 nM	1 nM	0 nM
XAV939, 1 μM	120 μl	30 μl	60 μl	30 μl	60 μl	30 μl	50 μl	0 μl
Assay Cocktail	0 μl	120 μl	60 μl	120 μl	60 μl	120 μl	50 μl	90 μl

Note: The concentrations of XAV939 are two times the desired working concentration because 25 μl of inhibitor assay cocktail is added per well and the final reaction volume is 50 μl. **Diluted inhibitor should be used immediately and any remainder discarded.**

6. Tankyrase 1 Enzyme

A. The kit contains 100 μl of Tankyrase 1 enzyme (Cat# 4700-096-01) at a concentration of 10 mU/μl. For the Tankyrase 1 standard curve, make serial dilutions of the Tankyrase 1 using 1X I-PAR Assay Buffer (0.1 mg/ml BSA), and make at least 100 μl of each dilution just before use (Table 3). The final concentrations are 10 mU/25 μl, 5 mU/25 μl, 2.5 mU/25 μl, 1 mUnit/25 μl, 0.5 mU/25 μl, 0.25 mU/25 μl, and 0.1 mU/25 μl. The standard curve requires 25 μl/well of each Tankyrase 1 dilution and each is performed in triplicate (see Figure 1).

B. Inhibitor studies require 5 mU (25 μl/well) Tankyrase 1; add 15 μl of 10 mU/μl Tankyrase 1 (4700-096-01) to 735 μl of 1X I-PAR Assay Buffer just before use. **Refer to step VI.A.4.**

Table 3: Tankyrase serial dilutions for example plate setup.

Units Tankyrase 10 μl	10 mU well	5 mU well	2.5 mU well	1 mU well	0.5 mU well	0.25 mU well	0.1 mU Well
Tankyrase 10 mU/ μl	10 μl	125 μl	125 μl	100 μl	125 μl	125 μl	100 μl
1X I-PAR Assay Buffer	240 μl	125 μl	125 μl	150 μl	125 μl	125 μl	150 μl

Note: Tankyrase Standard Curve utilizes 25 μl of each Tankyrase dilution. The XAV939 Inhibition Curve combines 25 μl of 5 mU/well Tankyrase with 25 μl of inhibitor assay cocktail. Using a reagent plate to make enzyme dilutions and dispensing with a multichannel pipet is recommended to minimize the variability in data as a consequence of the time required to add enzyme to each well. **Diluted enzyme should be used immediately and any remainder discarded.**

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Figure 1. Sample Plate Setup