TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

Tankyrase 1 Colorimetric Activity Assay

96 Tests

Cat# 4700-096-K

Colorimetric assay kit for candidate inhibitor screening and determination of IC_{50} values of Tankyrase 1 inhibitors.

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Table of Contents

I. Introduction

Trevigen's **Tankyrase 1 Colorimetric Activity Assay** is a highly sensitive colorimetric 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 organization[1]. 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 target[4].

Trevigen's **Tankyrase 1 Colorimetric Activity Assay** is ideal for the screening of Tankyrase 1 inhibitors and determining IC_{50} values. The **Tankyrase 1 Colorimetric 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 colorimetric signal. Thus, the conversion of blue substrate correlates with Tankyrase 1 activity. Important features of the assay include: 1) colorimetric, 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 Colorimetric 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
4684-096-P	Histone-Coated Natural Strip Well Plate, 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
4822-96-08	TACS-Sapphire™	10 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
5. 5% Phosphoric acid or 0.2 M HCl

Equipment:

1. Micropipettes and tips
2. Multichannel pipettor 10 - 200 µl
3. Incubator set at 25°C
3. Wash bottle or microstrip wells washer (optional)
4. 96-well colorimetric plate reader
5. Reagent plate (Nunc 262146 or equivalent) or microtubes

V. Reagent Preparation

1. Phosphate Buffered Saline (PBS) Wash Solution
Prepare 500 ml of 1X PBS.

2. PBS + 0.1% Triton® X-100 Wash Solution
Prepare 500 ml of 1X PBS containing 0.1% Triton® X-100.

3. 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 dH_2O. 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.

4. 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 µl per well. This equation may be used to formulate custom reactions:

	Volume (per well)
Assay Substrate	15 µl
1X I-PAR Assay Buffer (with or without inhibitor)	10 µl
Total volume	25 µl

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

A. For the standard curve, dilute 420 µl of Assay Substrate with 280 µl of 1X I-PAR Assay Buffer, and add 25 µl per well. **Refer to step VI.A.3.**

B. For the inhibitor assay cocktail, dilute 390 µl of Assay Substrate with 260 µl of 1X I-PAR Assay Buffer. Use this cocktail to serially dilute a prepared inhibitor stock. **Refer to step V.5.**

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 uM 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 µl 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 inhibitor assay cocktails 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.**

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 dH_2O 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. TACS-Sapphire™

TACS-Sapphire is a colorimetric substrate that turns blue in the presence of Horseradish Peroxidase (HRP). The addition of an equal volume of 0.2 M HCl or 5% phosphoric acid stops the reaction to generate a yellow color that is stable for up to 60 minutes and absorbs at 450 nm.

11. Histone-Coated Natural Strip Well Plate, I-PAR

The natural 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 (IC_{50}) 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.

1. Determine assay layout and controls:
i. Negative Control: A negative control without Tankyrase 1 should be prepared to determine background absorbance.
ii. Activity Control for Tankyrase 1 Inhibitor Study: 5 mUnits/well Tankyrase without inhibitors. These wells provide the 100% activity reference point.
iii. 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 µl, 5 mUnits/25 µl, 2.5 mUnits/25 µl, 1 mUnits/25 µl, 0.5 mUnits/25 µl, 0.25 mUnits/25 µl, and 0.1 mUnits/25 µl. Add 25 µl of each standard to triplicate wells (See Figure 1, columns 1-3).
iv. 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 µl, 200 nM/25 µl, 100 nM/25 µl, 20 nM/25 µl, 10 nM/25 µl, 2 nM/25 µl, and 1 nM/25 µl (See Figure 1, columns 4-6).

2. Remove the strip wells from the wrapper, and add 50 µl/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.

3. Prepare Tankyrase 1 standard curve assay cocktail (Section **V.4.A**) and inhibitor assay cocktail (Section **V.5**). Add 25 µl each to the corresponding wells (see Figure 1). The final reaction volume is 50 µl, so inhibitors should be at two times the desired working concentration, prior to assay.

4. Add 25 µl of Tankyrase 1 enzyme (prepared in Section **V.6.A**) to the wells without inhibitor and 25 µl of 5 mUnits/well Tankyrase 1 enzyme (prepared in Section **V.6.B**) to the wells containing inhibitor(s), as directed in Figure 1.

5. The final reaction volume is 50 µl:

	Volume	Order of Addition
Assay Cocktail (with or without inhibitor)	25 µl	1
Tankyrase 1 enzyme	25 µl	2
Total volume	50 µl	

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

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

B. Detection

1. Wash strip wells 2 times with **1X** PBS + 0.1% Triton® X-100 (200 µl/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.

2. Add 50 µl per well of diluted anti-PAR monoclonal antibody (prepared in Section **V.8**). Incubate at room temperature (25°C) for 30 minutes.

3. Wash strip wells 2 times with **1X** PBS + 0.1% Triton® X-100 (200 µl/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.

4. Add 50 µl per well of diluted goat anti-mouse IgG-HRP conjugate (prepared in Section **V.9**). Incubate at room temperature (25°C) for 30 minutes.

5. Wash strip wells 2 times with **1X** PBS + 0.1% Triton® X-100 (200 µl/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.

6. Add 50 µl per well of pre-warmed TACS-Sapphire™ colorimetric substrate and incubate, **in the dark**, for 15 minutes at room temperature (25°C). Stop the reactions by adding 50 µl per well of 0.2 M HCl or 5% Phosphoric Acid, and read the absorbance (Abs) at 450 nm.

VII. Data Interpretation

The typical colorimetric Tankyrase 1 standard curve and inhibition curve for the Tankyrase inhibitor XAV939 are provided below. Subtract the mean background absorbance from those of the experimental wells, and plot the Tankyrase Activity versus relative absorbance (RelativeA450) for each sample (Figure 2). The Tankyrase 1 standard curve is used to translate absorbance values to activity units of Tankyrase 1 and to provide a control for the dynamic range and sensitivity of the assay.

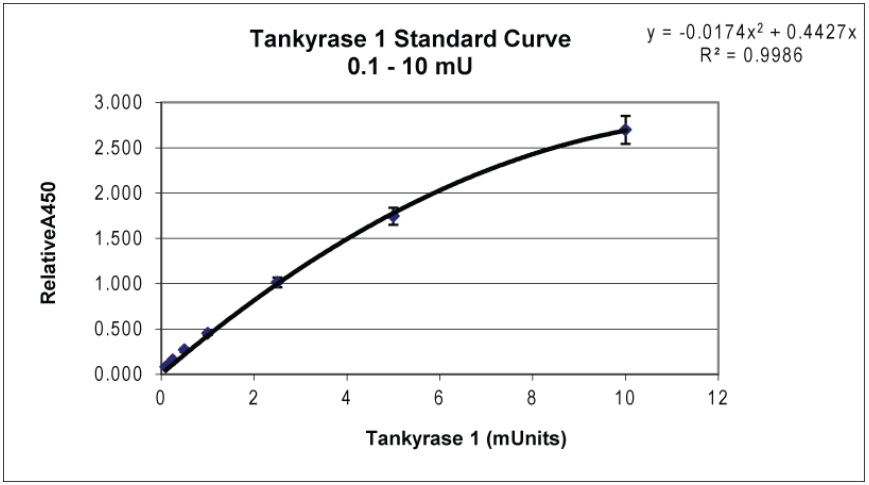

Figure 2. Graphical representation of the Tankyrase Standard Curve. The polynomial trendline generally provides the best fit curve. The line equation is used to translate absorbance values to mU of Tankyrase 1 for evaluating inhibitor efficiency.

The inhibition of Tankyrase 1 will be reflected as a decrease in the absorbance readings relative to that observed in the absence of inhibitors. Subtract the mean background from the experimental wells, and convert absorbance values for each sample to Tankyrase 1 activity units using the line equation from the standard curve. Subtract the sample activity units from the no inhibitor control to determine the units of inhibition for each well. Divide the units of inhibition for each well by the no inhibitor control and multiply by 100 to determine percent inhibition.

Step	Operation
1	Subtract mean background from each sample.
2	Absorbance is converted to Tankyrase 1 units using the line equation from the standard curve.
3	No inhibitor control units – sample units = inhibition units
4	Inhibition units / no inhibitor control units = inhibition ratio
5	Inhibition ratio x 100 = percent inhibition

Plot the inhibitor concentration versus the percent inhibition to create an inhibition curve. Once the inhibition curve is graphed, the linear portion of the curve should be identified and fit to a trendline (Figure 3).

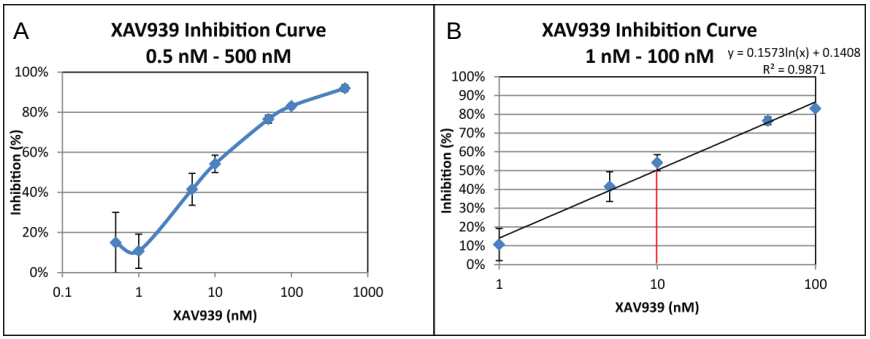

Figure 3. Graphical representation of a XAV939 Inhibition Curve. A) The Tankyrase 1 inhibitor, XAV939 inhibits 5 mU of Tankyrase 1 per well between 0.5 nM and 500 nM concentrations. B) By selecting the linear portion of the graph, the line equation may be determined by using a trendline.

Using the line equation (y = mx + b), the IC_{50} (x) can be calculated as:

				IC_{50} (nm)	
	y	**m**	**b**	**x**	**Published[3]**
XAV939	0.5	0.1573	0.1408	10	11

Figure 4. Calculated IC_{50} for XAV939.

VIII. Troubleshooting

PROBLEM	CAUSE	SOLUTION
No color in wells with Tankyrase 1 alone	If no color develops in the wells, then 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.
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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 Plate, I-PAR	1 plate
4701-096-K	Tankyrase 1 Chemiluminescent 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

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