Aurora Biolabs
High Purity Protein for Research

TR-FRET PARP1 Trapping Assay Kit

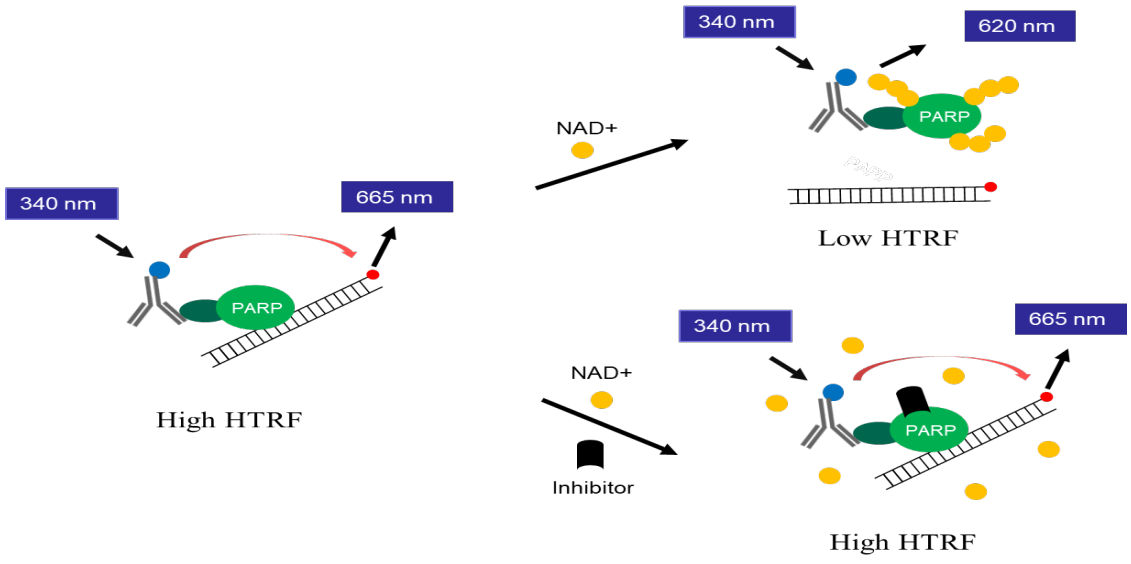
Catalog Number: 72771TAK

Background

PARP1 (Poly (ADP-ribose) polymerase 1) is an abundant member of the PARP family and plays a crucial role in DNA repair by acting as a damage sensor and facilitator. It binds to DNA at the site of damage, becomes catalytically activated, and uses NAD^+ as a substrate to add poly (ADP-ribose) (PAR) chains to itself and other proteins—a process called PARylation that results in the recruitment of other DNA repair proteins to the damaged site. Because of the high negative charge of PAR polymers, extensive autoPARylation of PARP1 leads to the dissociation of PARP1 from DNA, which is required for DNA repair completion. PARP1 is often overexpressed in various cancers, including breast, ovarian, prostate, lung, and glioblastoma. This overexpression is thought to support tumor cell survival. Some PARP inhibitors not only block the catalytic activity of PARP1 but also trap PARP1 on DNA at sites of damage, preventing its release. This creates a toxic DNA-protein complex that interferes with DNA replication and repair, leading to cell death—particularly in cancer cells deficient in homologous recombination repair (e.g., BRCA1/2-mutant cells).

Assay Principle

The TR-FRET PARP1 Trapping Assay Kit is designed to detect the poly-ADP-ribosylation activity of PARP1 and the status of PARP1 trapping on DNA. The DNA substrate in the kit is labeled with a fluorophore (acceptor). A Terbium (Tb)-labeled anti-Tag2 antibody binding to Tag2-Kras serves as the fluorescence donor. Activation of Tb results in fluorescence resonance energy transfer (FRET) if PARP1 binds to the fluorescence-labeled DNA, since the binding brings the fluorescence donor into close proximity with the fluorophore acceptor. Thus, the binding status can be quantitatively measured by calculating the ratio of the emission fluorescence intensities of the acceptor (665 nm) and the donor (620 nm). In the presence of NAD^+, auto-PARylation of PARP1 leads to dissociation of PARP1 from the DNA, resulting in a decrease in the FRET signal. Conversely, inhibition of auto-PARylation activity traps PARP1 on the DNA, and the FRET signal remains high.



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Application

High throughput screening of compounds that inhibit the auto-PARylation activity of PARP1 for drug discovery.

Plate Reader

A HTRF® certified microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is required.

Components

Catalog number	Item	Amount	Storage
7277-TAK-B	PARP Assay buffer	25 mL	-20°C
72771-T2P1	Recombinant human Tag2-PARP1	5 µL	-80°C
728429	4 mM NAD^+	40 µL	-20°C
4383866	Fluorescence-labeled DNA substrate	16 µL	-80°C
37882	Terbium-labeled anti-Tag2 antibody	20 µL	-80°C
	384-well microplate, White	1	Room temperature

Materials needed but not supplied

1. Microplate reader, HTRF® certified microplate reader
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

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Assay protocol

1. Prepare the inhibitor compound solution

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in assay buffer (since you will add 2 µl to the 20 µl reaction).

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare as series of further dilutions in assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

2. Prepare PARP1 solution

   Thaw PARP1 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C.

   Note: PARP1 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the PARP1 protein 500-fold (1 µL PARP1 + 499 µL assay buffer).

   Add 4 µl of diluted protein solution to each of positive control wells and inhibitor test wells.

   Add 4 µl of assay buffer to each of negative control wells.

3. Add inhibitor

   Add 2 µl of diluted compound solution to each inhibitor test well.

   Add 2 µl of inhibitor solvent solution to each of negative and positive control well.

4. Prepare the DNA substrate solution

   Dilute the fluorescence-labeled DNA 100-fold (1 µL DNA + 99 µL assay buffer).

   Add 4 µl of the diluted DNA solution to each well.

5. Prepare NAD^+ solution

   Dilute the NAD^+ 50-fold (1 µL NAD^+ + 49 µL assay buffer).

   Add 5 µl of diluted NAD^+ solution to each of positive control and compound test wells.

6. Prepare dye solution

   Dilute Terbium-labeled anti-Tag2 antibody 1:100. For example: 1 µl of Terbium-labeled anti-Tag2 antibody + 99 µl assay buffer.

Add 5 µl of this dye mixture to each well.

7. Incubate the reaction at room temperature for 30 minutes.
8. Measure fluorescent intensity

   HTRF compatible microplate reader is needed to measure fluorescent intensity of the samples.

   Fluorescent intensity should be measured twice:

   1. Excitation wavelength at 340 nm and emission at 620 nm.
   2. Excitation wavelength at 340 nm and emission at 665 nm.

Protocol Summary

Component	Negative Control	Positive Control	Inhibitor Test
Assay buffer	4 µl		
PARP1 protein		4 µl	4 µl
Inhibitor solvent	2 µl	2 µl	
Inhibitor solution			2 µl
DNA substrate	4 µl	4 µl	4 µl
NAD^+ solution		5 µl	5 µl
Assay buffer	5 µl		
Dye solution	5 µl	5 µl	5 µl
Total Volume	**20 µl**	**20 µl**	**20 µl**
Incubate at room temperature for 30 minutes.			

Data Analysis

1. Calculate sample HTRF signal of each well.

$$HTRF = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 620 nm}} X10{,}000$$

2. Calculate percentage activity

   In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\%\ \text{activity} = \frac{S - N}{\text{P} - \text{N}} X100$$

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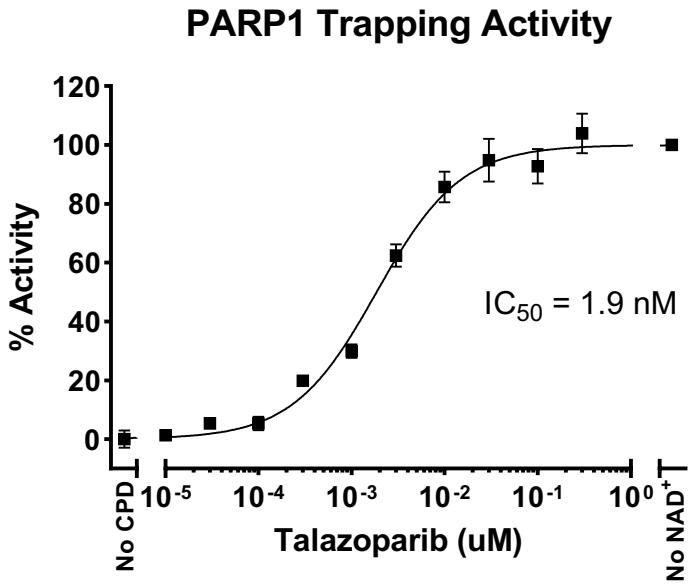
Assay result



Related products:

Product Name	Catalog #	Size
Recombinant Human PD-1	23731	100 µg
Recombinant Human PD-L1	237351	100 µg
Recombinant Human LAG3	235243	100 µg
Recombinant Human FGL1	233451	100 µg
Recombinant Human CD40	232340	100 µg
Recombinant Human CD40L	2323405	100 µg
Recombinant Human CD27	2323155	100 µg
Recombinant Human CD70	232370	100 µg
Recombinant Human OX40	236940	100 µg
Recombinant Human OX40L	2369405	100 µg
Recombinant Human GITR	234487	100 µg
Recombinant Human GITRL	2344875	100 µg
Recombinant Human CD40	232340	100 µg
Recombinant Human CD40L	2323405	100 µg
Recombinant Human CD155	2323155	100 µg
Recombinant Human TIGIT	2384448	100 µg
TEV Protease	190001	1,000 Units, 10,000 Units
TEV Protease- His-tag	190001-R	50 ug, 200 ug, 1 mg
PreScission Protease (HRV 3C)	190002	1,000 units, 10,000 units
Recombinant SUMO Protease (Ulp1)	190003	1,000 units, 10,000 units

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Recombinant YopH	200100	10 ug, 20 ug, 100 ug, 1 mg
Recombinant Biotin Protein Ligase (BirA)	90101	100 ug
Recombinant SortaseA-5M	90201	50 ug, 200ug
Recombinant Mouse Leukemia Inhibitory Factor	11-0001	10 ug, 100 ug
Recombinant Human LIF	12-0002	10 ug, 100 ug, 1 mg
Recombinant Human FGF-basic, Carrier-free	12-0005CFR	50 ug, 100 ug, 500 ug, 1 mg
Human SOS1, Avi-His tag	7671HA	50 µg, 100 µg
Kras WT Nucleotide Exchange Assay Kit	5727-4121NK	384 reactions
Kras G12C Nucleotide Exchange Assay Kit	5727-4122NK	384 reactions
Kras G12D Nucleotide Exchange Assay Kit	5727-4123NK	384 reactions
Kras G13D Nucleotide Exchange Assay Kit	5727-4133NK	384 reactions
Kras G12R Nucleotide Exchange Assay Kit	5727-4127NK	384 reactions
Kras G12V Nucleotide Exchange Assay Kit	5727-4128NK	384 reactions
Kras WT–cRAF Binding Assay Kit	5727-4121BK	384 reactions
Kras G12C–cRAF Binding Assay Kit	5727-4122BK	384 reactions
Kras G12D–cRAF Binding Assay Kit	5727-4123BK	384 reactions
Kras G12R–cRAF Binding Assay Kit	5727-4127BK	384 reactions
Kras G12V–cRAF Binding Assay Kit	5727-4128BK	384 reactions
Kras G13D–cRAF Binding Assay Kit	5727-4133BK	384 reactions
Kras WT/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4121CK	384 reactions
Kras G12C/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4122CK	384 reactions
Kras G12D/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4123CK	384 reactions
Kras G12V/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4128CK	384 reactions
Kras G13D/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4133CK	384 reactions
DNA Polymerase Theta Activity Assay Kit	362101	96 reactions, 384 reactions
OX40/OX40L Inhibitor Binding Assay Kit	2369401	384 reactions
PD-1/PD-L -1Inhibitor Binding Assay Kit	237352	384 reactions
T7 High Yield RNA Synthesis Kit	K777627	25, 50, 100 reactions
PKMYT1 Binding Assay Kit	756981BK	384 reactions
eIF4E/eIF4G Binding Assay Kit	34343BK	384 reactions
Caspase-3 Activity Assay Kit	810030	384 reactions
IDO1 Activity Assay Kit for Inhibitor Screening	910010	96 reactions
TEV Protease Activity Assay Kit	190001AK	96 reactions
SARS-CoV-2 Mpro (3CL Protease) Assay Kit	728203	96 reactions
SARS-CoV-2 Papain-like Protease Assay Kit	728253	96 reactions
SARS-CoV-2 Nucleocapsid Protein Binding Kit (For mouse antibody)	728263	384 reactions
SARS-CoV-2 Nucleocapsid Protein Binding Kit (For rabbit antibody)	728273	384 reactions

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