

PARP1 Histone H4 Activity Assay Protocol

Catalog #K611 Histone H4 96-well plate

Assay Description:

The Tulip Biolabs Cat. #K611 Histone H4 96-well plate is a microplate (Stripwell) coated with highly purified human histone H4 protein. The microplate wells are additionally treated with a proprietary blocking solution which stabilizes the plates for long-term storage at room temperature (RT), and minimizes background signal upon ELISA assay development. The #K611 plate is designed to be used in a PARP1 poly-(ADP-ribose) activity (PARylation) assay, as described below, or other uses as determined by the user. The described assay can be used to screen and measure IC50's of PARP1 enzyme inhibitors, or other uses. Total assay time is approximately 2.5 hours.

Summary of the Procedure:

The PARP1 Histone H4 Activity Assay Protocol describes a colorimetric assay based upon the PARP1 enzyme PARylation of the histone H4-coated 96-well microplate. In the procedure, 12.5µL aliquots of test samples (eg: PARP1 enzyme inhibitors, blanks, calibrators, quality control samples, and/or unknown samples) diluted in buffer are incubated with 12.5µL of PARP1/activated DNA in wells of the coated 96-well microplate. To start the PARylation reaction, 25µL of an NAD solution is added to a final concentration of 10 or 100µM. To stop the reaction, the microplate wells are washed with phosphate buffered saline (PBS). The plates are then immediately processed by ELISA by adding anti-poly(ADP-ribose), clone 10H ([Tulip Cat. #1020](#)) for 1h, washing in PBS, adding GAM-HRP for 30min, washing in PBS, then TMB chromogenic substrate is added. A blue color is developed over 15 minutes at RT, and then stopped by the addition of dilute acid stop solution. The final yellow intensity of the wells is measured at OD450nm in a microplate reader, and the absorbance is proportional to the extent of PARylation of the histone H4 in the sample well. The assay is generally easy to perform, low cost, and useful for screening PARP1 inhibitors. Please contact Tulip Biolabs to inquire about custom configuration and supply of the assay components according to your requirements.

Safety Warnings and Precautions:

The products described in this assay are for research use only (RUO) and are not approved for therapeutic or diagnostic use.

All research reagents can be considered as being potentially hazardous. We therefore recommend that the components of this described assay be handled only by qualified laboratory personnel who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately.

Materials Provided:

- HISTONE H4 96-WELL STRIPWELL PLATE (cat. #K611)

NOTE ON STORAGE: Store microplates at RT or 4°C. Place unused microwell strips back into the sealed foil pouch containing desiccant.

Additional Materials Required and Available from Tulip Biolabs:

- PARP1 enzyme, human, lyophilized ([cat. #2090](#))
- Anti-pADPr, clone 10H ([cat. #1020](#))

Additional Materials and Equipment Required:

Note: The sources of the reagents listed below have been used successfully, however alternative sources may be used.

- NAD (β-nicotinamide adenine dinucleotide, sodium salt; Sigma N0632; MW=685.4)
- DNA, activated (1mg/mL reconstituted in H₂O; calf thymus; Sigma D4522)
- 3-AB (3-aminobenzamide; Spectrum Chemical A3108; MW=136.2)
- Olaparib (Cayman Chemical 10621; MW=434.5)
- Anti-mouse IgG, HRP conjugate (GAM-HRP; Invitrogen A28177; Jackson ImmunoResearch 115-035-003)
- TMB substrate (Surmodics TMBW; 10mL per plate required)
- Stop solution, 0.2N HCl (10mL per plate required)
- Precision pipettes to deliver volumes of 12.5, 50, and 100µL.



- Multi-tip or repeating pipettes to deliver 100µl and/or 325µL (optional).
- Automated microplate washer (optional) or absorbant paper (paper towels) for blotting.
- Microplate reader capable of measuring OD450 nm.
- Microplate covers (e.g: Costar 3930 microplate cover) or sealing tape.

Buffer Solutions:

PARP Dilution Buffer (50mM Tris, pH7.4, 100mM NaCl, 10mM MgCl₂, 1mM TCEP)

2.5mL Tris (1M stock, pH7.4)

1.7mL or 0.29g NaCl (3M stock or MW=58.44)

0.5mL MgCl₂ (1M stock)

125µL TCEP (0.4M stock, pH7; 2.5µL/mL)

QS to 50mL

PBS (10mM sodium phosphate, 150mM NaCl, pH 7.4) for microplate washing.

PBST/1% BSA (10mM sodium phosphate, 150mM NaCl, pH 7.4, 0.1% Tween 20, 1% BSA) for primary and secondary antibody dilution.

Reagent Preparation:

PARP1 enzyme

Reconstitute lyophilized PARP1, human ([cat. #2090](#)) to 0.5mg/mL according to insert instructions. Aliquot 10µL per PCR tube, freeze quickly at -70°C, store in a sealed container (eg: 15mL conical centrifuge tube) at -70°C. Do not freeze/thaw (single-use). Stable for several months.

2X NAD solution (20µM or 200µM NAD)

5µL or 50µL NAD (20mM stock in 100mM Tris, pH7.4; 14mg/mL; Store single-use aliquots at -70°C.)

QS to 5mL in PARP Dilution buffer

4X PARP1/DNA solution (1µg/mL PARP1, 40µg/mL activated DNA)

3µL PARP1 (0.5mg/mL)[adjust quantity as necessary]

60µL DNA, activated (1mg/mL)

1.44mL PARP Dilution buffer

to make 1.5mL (1 plate)

4X Inhibitor solution (4mM 3-AB) [Dilute 1/10 in PARP Dilution buffer to make 400µM]

10µL 3-AB (400mM stock in EtOH; 54.5mg/mL)

990µL PARP Dilution buffer

to make 1mL

4X Inhibitor solution (4µM Olaparib) [Dilute 1/10 in PARP Dilution buffer to make 400nM]

3µL Olaparib (4mM stock in DMSO; 1.74mg/mL)

3mL PARP Dilution buffer

Final assay condition:

12.5ng/well PARP1, 10µg/mL activated DNA, 10 or 100µM NAD, +/-1000µM 3-AB or 1000nM Olaparib in PARP Dilution buffer

[Note: The quantity of PARP1/well may need to be adjusted according to the specific activity of the enzyme and desired control OD450 level]



Assay Procedure:

Note: During incubations, cover the microplate (e.g. Costar 3930 microplate cover or sealing tape).

1. Allow buffers to equilibrate to room temperature.
2. Prepare final 2X NAD, 4X PARP1/DNA, and 4X Inhibitor solutions just before use.
3. Add 12.5µL of 4X Inhibitor samples (make dilutions in PARP Assay buffer as desired; include blank buffer control) to wells of the microplate (#K611) as determined by the user (see example plate set-up).
4. Add 12.5µL of 4X PARP1/DNA solution (include blank buffer control) to wells of the microplate.
5. To start the reaction, add 25µL 2X NAD solution (10 or 100µM) per well (include blank buffer control). Tap each side of the plate to ensure thorough mixing.
6. Allow the plate to set for 30 minutes at room temperature (20-25°C).
7. Stop the PARylation reaction by washing the plate 4-times with 325µL of phosphate buffered saline per well with an automated microplate washer or manually using a multi-tip pipette. Be careful that the pipette tips do not touch the well bottoms.
8. Add 100µL/well of anti-pADPr, clone 10H (Tulip Cat. #1020) diluted 1/2K in PBST/1% BSA. Tap each side of the plate to ensure thorough mixing, then set for 1h at RT.
9. Wash as in step 7.
10. Add 100µL/well of GAM-HRP diluted 1/5K in PBST/1% BSA. Tap each side of the plate to ensure thorough mixing, then set for 30min at RT.
11. Wash as in step 7.
12. Add 100µl TMB SUBSTRATE to each well. Tap each side of plate to ensure thorough mixing.
13. Incubate at room temp for approximately 15 min, preferably in the dark or dim light. The wells will develop colorless to blue in color.
14. Add 100µL STOP SOLUTION to each well. **CAUTION: CONTAINS 0.2N HCl, A MILDLY CAUSTIC SOLUTION! AVOID CONTACT WITH SKIN AND EYES!** The wells will immediately change to a yellow color.
15. Read OD at λ 450nm in a microplate reader between 2 to 10 min after STOP SOLUTION addition. Be careful to eliminate any air bubbles in the wells before reading. Note: Read OD at λ 600nm and subtract from the OD450nm values well for well to adjust for light scattering (particulates, bubbles, etc), if desired.

Sample Analysis:

A cut-off value for OD450 can be used to determine the presence of PARP1 enzyme inhibition in test samples. Recommended standards are as follows, however the user must determine appropriate values:

StdBlank: PARP1/DNA, no NAD.

StdCutoff (IC50's): 7.5nM Olaparib/10µM NAD or 55nM Olaparib/100µM NAD; 10µM 3-AB/10µM NAD or 100µM 3-AB/100µM NAD prepared in PARP Dilution buffer.

Additional Assay Considerations:

- For primary and secondary antibody dilution buffers, Blotto (PBST/1% Carnation Instant Milk) can be substituted for the PBST/1% BSA. The results with Blotto show lower control background levels, however the overall signal is also reduced requiring increased incubation time in TMB (30 min) and/or higher amounts of PARP1.
- The quantity of PARP1/well and/or length of the rxn time may need to be adjusted according to the specific activity of the PARP1 (due to lot differences, handling/storage, etc). Generally, an OD450 of 1.0 to 2.0 is the target level for 100% activity.
- Verification of the assay under the user's conditions can be made by comparing measured IC50's of 3-AB and/or Olaparib with the results reported herein (please see the attached example data).
- Contact Tulip Biolabs for additional information/suggestions.

Limitation on use:

The products and materials describe herein are for research use only (RUO) and are not approved for therapeutic or diagnostic use. In all circumstances, the user must validate the method for suitability of their intended purpose.

Related References:

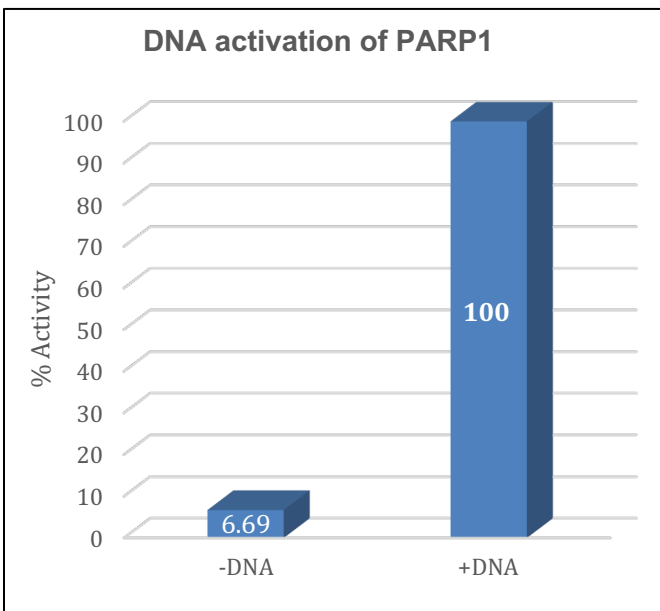
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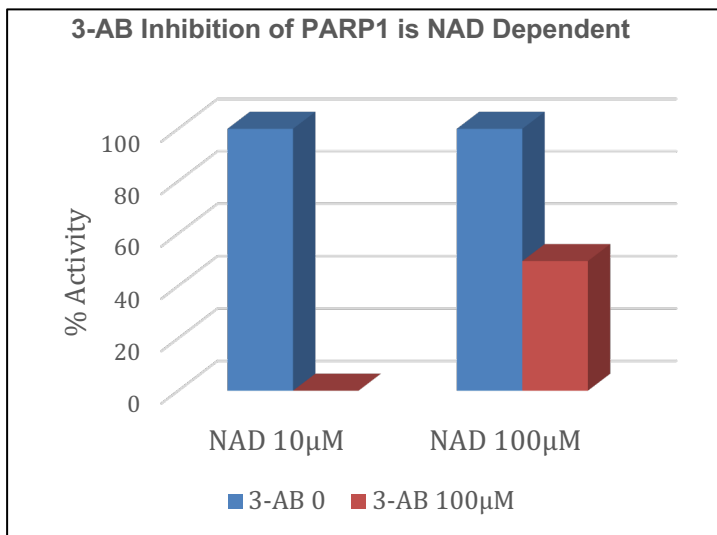


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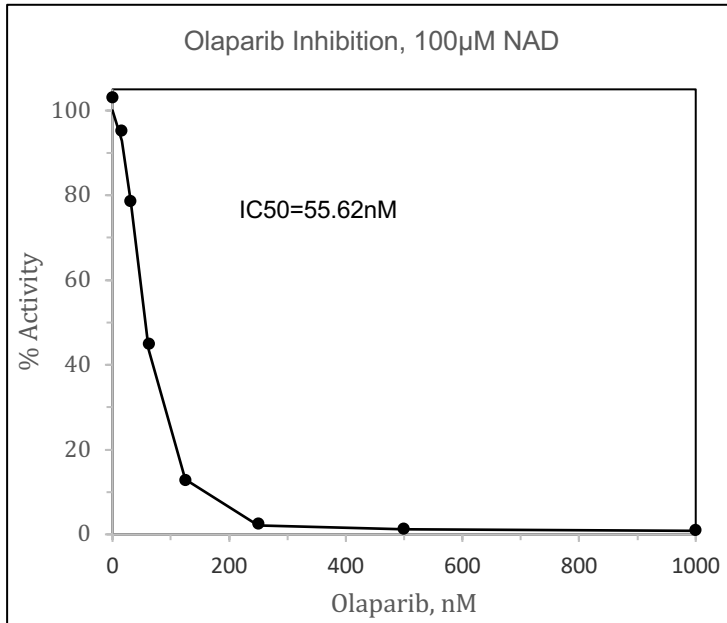
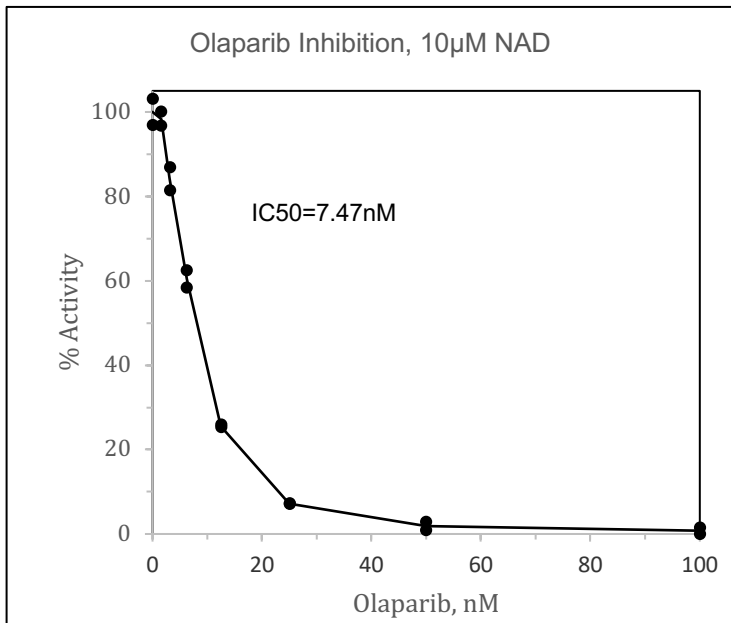
Example results:



PARP1 Histone H4 microplate assay was performed with 10µM NAD either with 10µg/mL activated/nicked DNA (+DNA) or without (-DNA). The addition of DNA increases the relative activity (corrected OD450) from 6.69% to 100%, approximately 15-fold. 100% activity corresponds to OD450corr = 1.901.



PARP1 Histone H4 microplate assay was performed with 10µM and 100µM NAD either with or without 100µM 3-aminobenzamide (3-AB). The extent of 3-AB inhibition is competitive with NAD. 100% activity corresponds to OD450corr = 1.901 and 2.144 for 10 and 100µM NAD, respectively.



PARP1 Histone H4 microplate assay was performed at 10µM NAD (left) and 100µM NAD (right) with increasing concentrations of Olaparib, as indicated (note the concentration scale difference). The IC₅₀ for Olaparib at 10µM NAD is 7.47nM, and at 100µM is 55.62nM. Thus, the Olaparib inhibition is competitive with NAD in this assay. 100% activity corresponds to OD450corr = 1.604 and 1.800 for 10 and 100µM NAD, respectively.



Example of plate set-up (Note: The user must determine the appropriate layout for their purpose.)

	10 μ M NAD		100 μ M NAD		Controls							
<>	1	2	3	4	5	6	7	8	9	10	11	12
A	100nM Olaparib		1000nM Olaparib		no NAD							
B	50nM		500nM									
C	25nM		250nM		no NAD +inhibitor							
D	12.5nM		125nM									
E	6.25nM		62.5nM		no PARP1 /DNA + NAD							
F	3.125nM		31.25nM									
G	1.5nM		15nM		Buffer only							
H	0		0									

Controls:

no NAD=PARP1/DNA, no NAD

no NAD + inhibitor=PARP1/DNA, no NAD, 1000nM Olaparib

no PARP1/DNA +NAD=no PARP1/DNA + 100 μ M NAD

Buffer only=only PARP Dilution buffer

