Signosis
Innovative Plate Assay Solutions

PARP Activity Assay Kit

Catalog Number EA-7063 **(For Research Use Only)**

Introduction

Poly(ADP-ribose) polymerase (PARP) is a crucial protein that is involved in repairing DNA. This protein detects single-strand DNA breaks and signals the enzymatic machinery needed for DNA repair. Specifically, PARP binds to the DNA and, using NAD+ as a substrate, synthesizes a polymeric adenosine diphosphate ribose chain, which acts as a signal for DNA=repairing enzymes. PARP is a clinically significant enzyme, because suppressing its activity with inhibitors have been shown to kill cancer cells. Because of this PARP inhibitors have been used to treat a large number of different cancers, including breast, prostate, and pancreatic cancer.

Principle of the assay

The PARP activity assay kit measures the activity of PARP by detecting its ability to perform ADP-ribosylation on histones. First, a plate is coated with histone. Then, a mixture of activated DNA and a biotin-tagged PARP substrate (NAD+) is added to the wells. When samples containing PARP are added to the mixture, the PARP will attach the biotin-tagged substrate to the histone through ADP-ribosylation. Once the wells are washed, the biotin-tagged substrate, which is bound to the histones attached to the plate, can be detected using streptavidin-HRP. After the addition of HRP substrate and stop solution, a colorimetric signal is generated, which can be measured at an absorbance of 450 nm with a plate reader.

Materials Provided

- 1x Diluent Buffer (4°C)
- 5x Assay Wash Buffer (4°C)
- Histone Stock (-80°C)
- Activated DNA (-80°C)
- PARP Substrate (-80°C)
- HRP Reagent (4°C)
- TMB Substrate (4°C)
- Stop Solution (4°C)

Reagent preparation before starting experiment

- Dilute the 5x Assay Wash Buffer 1:5 with ddH2O to make 1x Wash Buffer
  - 40 ml 5x Assay Wash Buffer
  - 160 ml ddH2O .
- Dilute HRP Reagent 1:200 with 1x Diluent buffer.

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

Assay Procedure

1. Make Histone solution by diluting the Histone stock 1:1000 in PBS.
2. Coat the plate by adding 100 µL of Histone solution to each well.
3. Incubate the plate at room temperature for at least 2 hours or at 4°C overnight for optimal results.
4. Discard the histone solution in the plate and wash the plate by aspirating and washing each well with 200µl of 1x Assay Wash Buffer. Repeat the process three times for a total of three washes.
5. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
Activated DNA	0.05 µL
PARP Substrate	0.5 µL
PBS	49.45 µL
Total	50 µL

6. Add 50 µL of reaction mix to each well of the plate.
7. Add 50 µL of sample to each well with reaction mix and mix thoroughly.
8. Incubate the plate at room temperature for 1 hour.
9. Make HRP solution by diluting the HRP reagent 1:200 in 1x Diluent Buffer.
10. Discard the contents in the plate and wash the plate by aspirating and washing each well with 200µl of 1x Assay Wash Buffer. Repeat the process three times for a total of three washes.
11. Add 100 µL of HRP solution to each well of the plate.
12. Incubate the plate at room temperature for 1 hour.
13. Discard the HRP solution in the plate and wash the plate by aspirating and washing each well with 200µl of 1x Assay Wash Buffer. Repeat the process three times for a total of three washes.
14. Add 100 µL of TMB substrate to each well of the plate.
15. Incubate the plate at room temperature for 30-60 minutes until the wells begin to turn blue.
16. Once the desired color is reached, add 50 µL of Stop solution to each well of the plate to terminate the HRP reaction and form a yellow color.
17. Using a microplate reader, measure the absorbance of the plate at 450 nm.