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

- Histone Stock (-80°C)
- Activated DNA (-80°C)
- PARP Substrate (-80°C)
- HRP Reagent (4°C)
- TMB Substrate (RT)
- Stop Solution (RT)

Sample preparation before starting experiment

- For **cell culture medium samples**, add 50µl directly to the well.
- For **cell lysate samples**, use lysis buffer. Cell Lysis Buffer for ELISA available (Catalog# EA-0001). Follow protocol in Cell Lysis Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X Diluent buffer, for example, add 80ul sample in 720ul 1X Diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Assay Procedure

1. Make Histone solution by diluting the Histone stock 1:2000 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.
4. Discard the histone solution in the plate and wash the plate twice with PBS.
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	1 µL
PARP Substrate	1 µL
PBS	48 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 PBS.
10. Discard the contents in the plate and wash the plate three times with PBS.
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 three times with PBS.
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.