Automodified PARP1 96-well Plate Catalog #K622

LIMITATIONS: THIS PRODUCT IS FOR RESEARCH USE ONLY AND IS NOT APPROVED FOR THERAPEUTIC OR DIAGNOSTIC USE.

THE FOLLOWING INFORMATION IS INTENDED ONLY AS A GUIDE. THE USER MUST VALIDATE THE EXPERIMENTAL CONDITIONS FOR SUITABILITY OF THEIR INTENDED PURPOSE.

Description:

The Tulip Biolabs Cat. #K622 Automodified PARP1 96-well plate is a microplate (Stripwell) coated with highly purified automodified (PARylated) human PARP1 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 Cat. #K622 plate is intended to be used in a PARG glycohydrolase assay, as described in a separate protocol, or other uses as desired.

General Method:

Purified PARG, target glycohydrolase, or prepared cell extracts are added to wells of the #K622 Automodified PARP1 plate. Following incubation, the plates are developed by ELISA for remaining poly(ADP-ribose)(PAR) using an appropriate antibody (eg: Cat. #1020, see the attached protocol).

Supplied As:

The Tulip Biolabs Cat. #K622 Automodified PARP1 96-well plate is supplied as a removable strip 96-well microplate coated with highly purified human automodified (PARylated) PARP1 protein (Cat. #2095). The microplate wells are additionally treated with a proprietary blocking solution which stabilizes the plates for long-term storage at room temperature (RT). Each microplate is supplied in a foil pouch with desiccant, and sealed under slight negative pressure.

Storage and Stability:

Store microplates at RT or 4˚C. Place unused microwell strips back into the sealed foil pouch containing desiccant. Microplate strips are stable for at least 6 months at RT.

Suggested Applications:

Measure PARG, other enzymes, or cell extracts for PAR glycohydrolase activity.

Tulip BioLabs Other Related Products:

Histone H4 96-well microplate, Cat. #K611.
PARP1, Highly active, human, Cat. #2090.
PARP1, Automodified, human, Cat. #2095.
Anti-poly(ADP-ribose) polymer, clone 10H, mouse monoclonal antibody, Cat. #1020.
Anti-poly(ADP-ribose) polymer, IgY, chicken polyclonal antibody, Cat. #1023.

Original Reference:

This product was developed at Tulip Biolabs, Inc.

Related General References:

Nie, L *et al*. 2023. eLife 12: RP89303
Wondisford AR *et al*. 2024. Nat Struct Molec Biol 31: 791

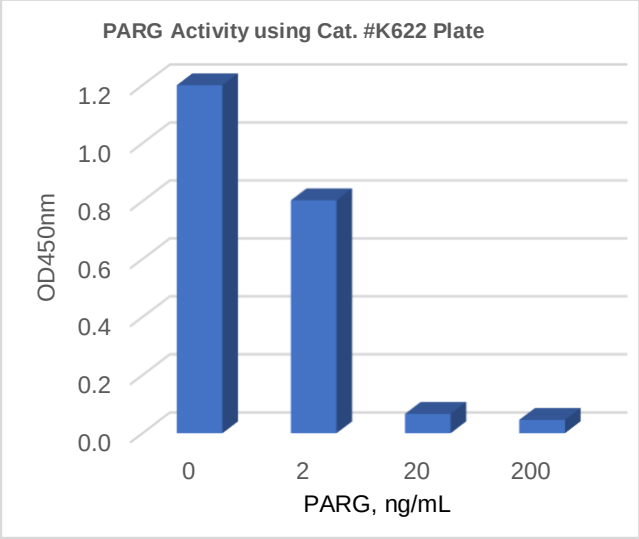

Figure. PARG dePARylates Cat. #622 Plates. Human PARG (full-length, recombinant) at 0, 2, 20, and 200ng/mL were added to the wells of the automodified PARP1 96-well microplate (Cat. #K622) and allowed to incubate for 1h at RT. The wells were washed with PBS, then processed by ELISA using anti-pADPr (Cat. #1020) then GAM-HRP. Colorimetric development used TMB for 15min then stop solution added. The OD450nm was measured in a microplate reader. The graph shows a PARG concentration dependent decrease in OD450, signifying the cleavage of PAR from the automodified PARP1 on the plate.

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EXAMPLE PROTOCOL to Test PARG Activity using Automodified PARP1 96-well Plate, Catalog #K622

MATERIALS REQUIRED

PARG enzyme, recombinant (prepared by Tulip Biolabs)
Automodified PARP1 96-well Plate (Cat. #K622)
PARG Assay buffer (PBS, 0.1mM DTT)
Anti-pADPr, clone 10H (Cat. #1020)
PBST/2% Blotto (PBS, 0.1% Tween 20, 2% Carnation non-fat dry milk)
Goat anti-mouse IgG conjugated HRP (Jackson ImmunoResearch)
TMB substrate
Stop solution (0.2N HCl)

Assay Procedure:

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

1. Allow buffer and components to equilibrate to room temperature (RT).
2. Dilute PARG to the desired concentration in PARG Assay buffer, for example 2, 20, 200ng/mL.
3. Add 50µL of 2X inhibitor samples (make dilutions in PARG Assay buffer) or other test samples as desired; include blank buffer control to wells of the microplate (Cat. #K622) as determined by the user.
4. Add 50µL PARG solution per well to start the reaction. Include blank buffer controls.
5. Allow the plate to incubate for 30-60 minutes at room temperature (20-25˚C), or as desired.
6. Stop the dePARylation reaction by washing the plate 2-times with 325µL phosphate buffered saline (PBS) per well using an automated microplate washer or manually with a multi-tip pipette.

Microplate Development:

7. Add 100µL/well of anti-pADPr, clone 10H (Cat. #1020) diluted 1/2K in PBST/2% Blotto. Tap each side of the plate to ensure thorough mixing, cover then set for 2h at RT.
8. Wash the microplate 4-times with 325µL PBS per well using an automated microplate washer or manually with a multi-tip pipette.
9. Add 100µL/well of GAM-HRP diluted 1/5K in PBST/2% Blotto. Tap each side of the plate to ensure thorough mixing, cover then set for 45min at RT.
10. Wash as in step 8.
11. Add 100µl TMB substrate to each well. Tap each side of plate to ensure thorough mixing.
12. Cover, then incubate microplate at RT for 10-30min, preferably in the dark or dim light. The wells will develop from colorless to blue in color.
13. 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.
14. Read OD at λ450nm 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 λ600nm and substract from the OD450nm values well for well to adjust for light scattering (particulates, bubbles, etc), if desired.

Limitation on use:

This product is for research use only (RUO) and is not approved for therapeutic or diagnostic use. In all circumstances, the user must validate the method for suitability of their intended purpose.

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