

ADHP Assay Stop Reagent

CATALOG NUMBER: PRB-5219

QUANTITY: 50 mg (250 reactions)

Background

Assays that employ 1-(3,7-dihydroxy-10H-phenoxazin-10-yl)-ethanone (ADHP) provide a sensitive method of detecting enzymatic systems that generate hydrogen peroxide. Hydrogen peroxide reacts with ADHP in the presence of horseradish peroxidase (HRP) to generate the colorimetric/fluorescent product resorufin.

Cell Biolabs' ADHP Assay Stop Reagent provides several improvements over traditional ADHP assay measurements:

- Allows for user control of reading results by allowing the colorimetric/fluorescence reaction to be stopped at an optimal time point.
- Stabilizes output signal for up to three hours at room temperature, allowing user to have time flexibility in reading results
- Prevents over production of resorufin that can lead to misleading "signal burnout" results.

Cell Biolabs' proprietary ADHP Assay Stop Reagent is provided as a solid that is easily reconstituted to a solution. Subsequent to addition of the solution, the assay may be read on a plate reader immediately or up to three hours later. The provided solid is enough to stop 250 reactions of 100 μ L each.

Storage

Store solid reagent or reconstituted solution at -20°C protected from light.

Shelf Life

Solid reagent: One year from date of receipt under proper storage conditions.

Reconstituted reagent: One month from date of reconstitution under proper storage conditions.

Protocol:

1. Warm up the ADHP Assay Stop Reagent at room temperature.
2. Prepare 20 mg/mL of ADHP Assay Stop Reagent in 100% Ethanol in a 2 mL screwcap tube. Weigh out a minimum of 5 milligrams. For 5 milligrams of solid, transfer the solid to a 2 mL screwcap tube.
3. Reconstitute solid by mixing 5 milligrams well with 0.25 mL of 100% Ethanol until all of the solid is dissolved. Scale the volume of Ethanol as needed based on the weight of the solid.
4. To make the final ADHP Assay Stop Solution, add an equal volume of distilled water to the 20 mg/mL ADHP Assay Stop Reagent tube and mix well. The final concentration of ADHP Assay Stop Solution contains 10 mg/mL of ADHP Assay Stop Reagent in 50% Ethanol.

Note: 1.0 mL of ADHP Assay Stop Solution is enough to stop 50 ADHP reactions of 100 μ L each. Scale up proportionally if more stop reactions are required.

- At the desired ADHP assay stopping time point, add 20 μ L of ADHP Assay Stop Solution per 100 μ L reaction sample well and read the plate in a plate reader. The fluorescence signal remains stable for at least three hours.
- Store any unused ADHP Assay Stop Solution at -20°C .
Note: The ADHP Assay Stop Solution is stable at -20°C protected from light for up to one month.

Example of Results

The following figures demonstrate typical results. One should use the data below for reference only. This data should not be used to interpret actual results.

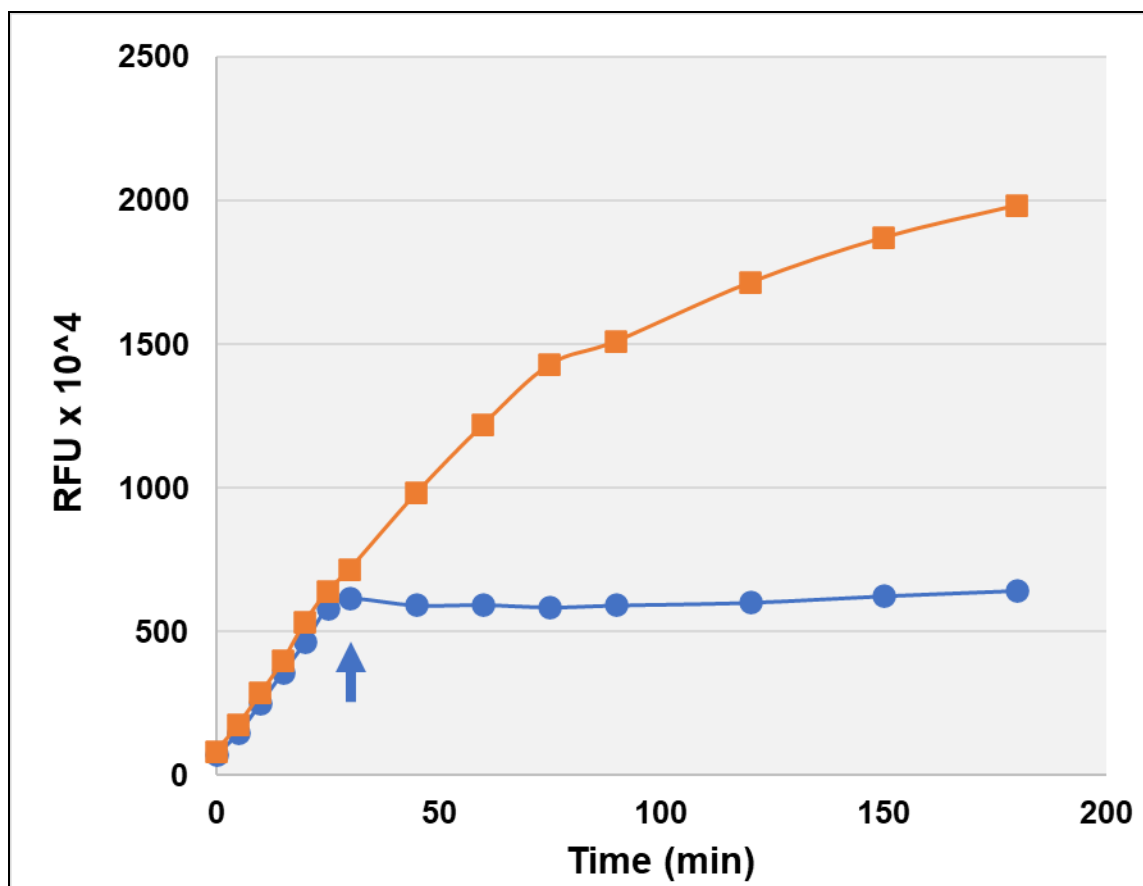


Figure 1: ADHP Assay Stop Reagent stops progression of ADHP-based Cholesterol assay. 10 μ M Cholesterol was used in a Total Cholesterol Assay. After 30 min reaction at 37°C (indicated by blue arrow), ADHP Assay Stop Solution was added to one of the reactions (blue line) and not to the other (orange line) and then incubated at 37°C for various times.

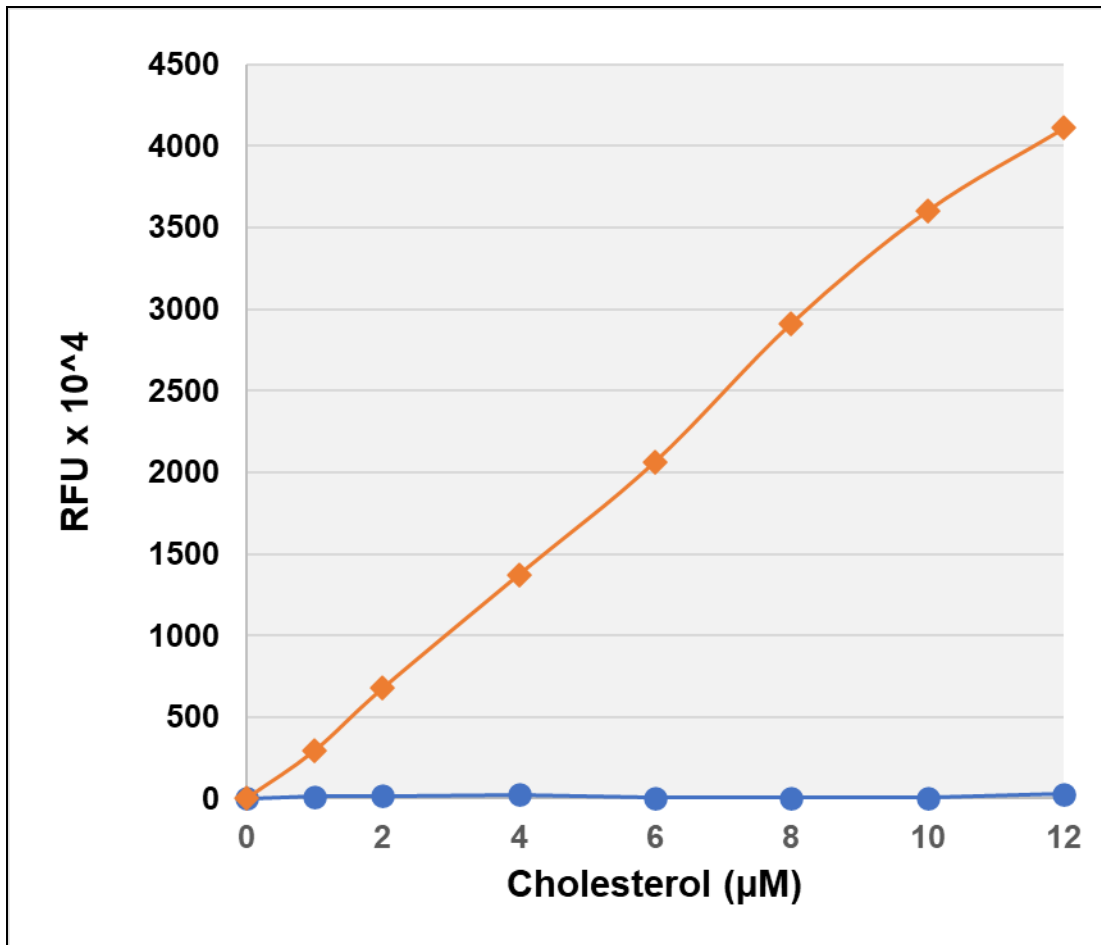


Figure 2: ADHP Assay Stop Reagent prevents ADHP conversion to fluorescent product. A Total Cholesterol Assay was performed in absence (orange line) or presence (blue line) of ADHP Assay Stop Solution for 60 minutes at 37°C.

Warranty

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This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

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