Hyaluronidase Inhibitor Screening Assay Kit (Colorimetric)

ARG83000 Hyaluronidase Inhibitor Screening Assay Kit is a detection kit for the quantification of Hyaluronidase Inhibitor Screening in Test Compounds, Hyaluronidase Inhibitors samples.

Catalog number: ARG83000

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Hyaluronidase is an enzyme that depolymerizes the mucopolysaccharide hyaluronic acid, which is a component of the mucoprotein ground substance or tissue cement and thus increases membrane permeability, reduces viscosity, and makes tissues more readily permeable. It is found in snake venoms and in the venoms of Hymenoptera, such as honey bees and yellow jacket wasps. It is available for therapeutic use in animal-derived formulations and as a human recombinant form.

PRINCIPLE OF THE ASSAY

This Hyaluronidase Inhibitor Screening Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Hyaluronidase Inhibitor in all compounds that affect hyaluronidase activity samples. The Hyaluronidase Inhibitor Screening Assay kit uses a two-step turbidimetric reaction to measure hyaluronidase activity by the amount of hyaluronic acid that is hydrolyzed. A stop reagent halts the enzymatic reaction and forms turbidity with any residual hyaluronic acid in the well. The decrease in turbidity at 600 nm is directly proportional to hyaluronidase activity in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped with blue ice. Store all components at-20°C upon receiving.

Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Enzyme Buffer	5 mL	-20°C
Assay Buffer	5 mL	-20°C
Substrate	1.5 mL	-20°C
Stop Reagent	20 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 600 nm
- Centrifuge
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- 1%-5% DMSO

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE PREPARATION

- Enzyme Preparation: Hyaluronidase should be prepared in Enzyme Buffer and used fresh. Albumin and other proteins interfere with this assay and should not be included in the Enzyme Buffer
- Determine Optimal Hyaluronidase Concentration for Inhibitor Screening:

  1. Prepare stock Hyaluronidase solution in Enzyme Buffer (start at a high concentration, you can always dilute down). In eppendorf tubes, serially dilute 50 µL of stock Hyaluronidase in Enzyme Buffer.

  2. Transfer 40 µL of each Hyaluronidase dilution into separate wells of a clear, flat bottom 96-well plate

  3. Transfer 40 µL of dH2O into two separate wells for the No Enzyme Control (NEC) and No Substrate Control (NSC).

  4. Prepare enough Working Reagent for each well by combining 10 µL Substrate and 35 µL of Assay Buffer. Add 40 µL Working Reagent to

sample wells and the NEC. Add 40 µL Assay Buffer to the NSC well.

6. Tap plate briefly to mix and incubate the plate for 20 minutes at room temperature.

7. Add 160 µL Stop Reagent to each well. Tap plate to mix briefly and thoroughly.

8. Incubate for 10 minutes at room temperature and read optical density at 600 nm.

- Inhibitor Preparation: Dissolve the inhibitors in solvent of choice. DMSO at concentrations of 1 v/v% or less in the 100 µL enzymatic reaction will not interfere (the 20 µL of test compounds may be in 5% DMSO).

REAGENT PREPARATION

- Working Reagent: prepare fresh Working Reagent for each reaction by mixing 10 µL Substrate and 35µL Assay Buffer.

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

1. For each inhibitor and inhibitor concentration being tested, transfer **40 µL** of **hyaluronidase** into separate wells of clear bottom 96-well microplate.
2. Transfer an additional **40 µL** of **hyaluronidase** and **40 µL** of **Enzyme Buffer** into separate wells for the No Inhibitor Control (NIC) and No Enzyme Control (NEC) respectively.
3. Add **20 µL** of the solvent (use with the test compound) into the NIC and NEC wells.
4. Add **20 µL** of each respective test compound into the sample wells.
5. Incubate for **15 minutes** at **room temperature.**
6. Add **40 µL** of **Working Reagent** into each wells. Tap plate to mix.
7. Incubate for **20 minutes** at **room temperature**.
8. Add **160 µL** of **Stop Reagent** to each well.
9. Incubate for **10 minutes** at **room temperature**. Read the absorbance at **O.D. 600 nm**.

CALCULATION OF RESULTS

1. Calculate the % inhibition is computed as follows:

% Inhibition = [1- ($OD_{No\ Enzyme}$ – $OD_{Test\ Compound}$) / ($OD_{No\ Enzyme}$ – $OD_{No\ Inhibitor}$)] x 100%

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Hyaluronidase Inhibitor Screening Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.