



Hyaluronidase Inhibitor Screening Assay Kit

Catalog Number KA6201

100 assays

Version: 03

Intended for research use only

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Introduction

Background

HYALURONIDASES are a family of enzymes that catalyze the degradation of the glycosaminoglycan, hyaluronic acid. Hyaluronic acid is one of the major constituents of the extracellular matrix in organisms where it contributes to both cell proliferation and migration. The role of hyaluronidases in breaking down this key factor in cell proliferation makes them a possible target for cancer treatment. One hypothesis is that increased hyaluronidase may help prevent tumor invasion by breaking down the extracellular matrix needed for tumor expansion. Conversely, decreasing hyaluronidase activity might prevent metastasis by stopping cancer cells from escaping primary tumor masses. The study to determine hyaluronidases' exact role in cancer pathology is still ongoing.

Principle of the Assay

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.

- Applications:
 - ✓ High-throughput screening for evaluation of hyaluronidase inhibitors.

- Key Features:
 - ✓ Rapid and reliable. Entire assay can be completed in 30 min.
 - ✓ Simple and Convenient. Simple procedure with an enzymatic reaction and addition of stop reagent. No wash or reagent transfer steps involved.
 - ✓ Robust and amenable to HTS. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

General Information

Materials Supplied

List of component

Component	Amount
Substrate	1.5 mL
Stop Reagent	20 mL
Assay Buffer	5 mL
Enzyme Buffer	5 mL

Storage Instruction

The kit is shipped at RT. Store kit components at -20°C upon receipt. Shelf life: 12 months after receipt.

Materials Required but Not Supplied

Purified Human HAase or Bovine HAase and if desired a control HAase inhibitor, pipetting devices, eppendorf tubes, clear flat-bottom 96-well plates, and plate reader.

Precautions for Use

- Precautions
- ✓ 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.

Assay Protocol

Reagent Preparation

Prior to assay, equilibrate all components to room temperature. The Working Reagent should be prepared fresh and used within two hours.

✓ **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.

The following protocol is optimized for Human Hyaluronidase or Bovine Hyaluronidase. If using a different species or brand of enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well.

Dilute human hyaluronidase to 20 U/mL in Enzyme Buffer. If using bovine hyaluronidase, dilute to 10 U/mL in Enzyme Buffer.

Sample Preparation

✓ **Test Compound Preparation:**

Dissolve the test compounds (i.e. inhibitors) in solvent of choice. It is prudent to first test the tolerance of the solvent by the enzyme of choice. DMSO at concentrations of 1 v/v% or less in the 100 μ L enzymatic reaction will not interfere (the 20 μ L of test compounds may be in 5% DMSO).

Assay Procedure

This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Note: Neither the enzyme Hyaluronidase nor a control inhibitor is included in the kit.

✓ **Inhibitor Screening Procedure:**

1. For each inhibitor and inhibitor concentration being tested, transfer 40 μ L of hyaluronidase into separate wells of a 96-well plate.
2. Transfer an additional 40 μ L of hyaluronidase and 40 μ L Enzyme Buffer into separate wells for the No Inhibitor Control (NIC) and No Enzyme Control (NEC) respectively.
3. To the NIC and NEC wells, add 20 μ L of the solvent in use with the test compounds.
4. To the sample wells, add 20 μ L of each respective test compound.
Note: the concentration of test compound in the 20 μ L should be 5 $\times$ the desired final concentration in the 100 μ L enzymatic reaction.
5. Incubate wells with test compounds for 15 min at room temperature.
6. Prepare enough Working Reagent for each well by combining 10 μ L Substrate and 35 μ L Assay Buffer.
Add 40 μ L Working Reagent to every well and tap plate to mix immediately.
7. Incubate the plate for 20 minutes at room temperature.
8. Add 160 μ L Stop Reagent to each well. Tap plate to mix briefly and thoroughly.
9. Incubate for 10 minutes at room temperature and read optical density at 600 nm.

Note: this assay can be run in 384-well plates by using $\frac{1}{4}$ of the volumes for 96-well plate assay.

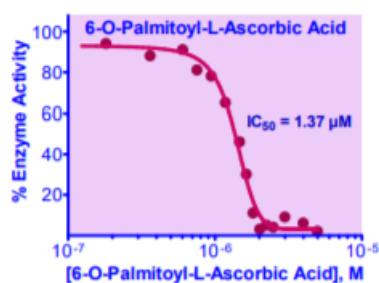
Data Analysis

Calculation of Results

Calculate the % inhibition using the equation below:

$$\% \text{ Inhibition} = \left(1 - \frac{OD_{\text{No Enzyme}} - OD_{\text{Test Cpd}}}{OD_{\text{No Enzyme}} - OD_{\text{No Inhibitor}}} \right) \times 100\%$$

Where $OD_{\text{No Enzyme}}$, $OD_{\text{No Inhibitor}}$, and $OD_{\text{Test Cpd}}$ are the optical density values of the No Enzyme Control, No Inhibitor Control, and Test Compound.



6-O-Palmitoyl-L-Ascorbic Acid Titration:

Recombinant human HAase was incubated with various concentrations of 6-O-Palmitoyl-L-Ascorbic Acid. Each concentration of inhibitor contained 5v% DMSO (final 1v% in 100 μL enzymatic reaction).

Resources

References

1. Lokeshwar, B., et al (2005). HYAL1 Hyaluronidase in Prostate Cancer: A Tumor Promoter and Suppressor. *Cancer Res* 65(17):7782-7789.
2. Girish, K., et al (2009). Hyaluronidase Inhibitors: A Biological and Therapeutic Perspective. *Curr. Med. Chem.* 16(18):2261-2288.
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4. Whatcott, C., et al (2016). Hyaluronidase To Enhance NanoparticleBased Photodynamic Tumor Therapy. *Nano Lett.* 16(4):2512-2521.

Plate Layout

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