QuantiChrom™ Hyaluronidase Inhibitor Screening Assay Kit (DIHY-100)

Rapid Turbidimetric Screening for Hyaluronidase Inhibitors

DESCRIPTION

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.

BioAssay Systems' 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.

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.

APPLICATIONS

High-throughput screening for evaluation of hyaluronidase inhibitors.

KIT CONTENTS (100 tests in 96-well plates)

Substrate:	1.5 mL	**Stop Reagent:**	20 mL
Assay Buffer:	5 mL	**Enzyme Buffer:**	5 mL

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

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.

ENZYME ACTIVITY 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.

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 (Acro Biosystems Cat # PH0-H5225) or Bovine Hyaluronidase (Calzyme Cat # 091A0300). If using a different species or brand of enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well (see Product FAQ on website for instructions).

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

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 µL enzymatic reaction will not interfere (the 20 µL of test compounds may be in 5% DMSO).

Inhibitor Screening Procedure:

1. For each inhibitor and inhibitor concentration being tested, transfer 40 µL of hyaluronidase into separate wells of a 96-well plate.
2. Transfer an additional 40 µL of hyaluronidase and 40 µL 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 µL of the solvent in use with the test compounds.
4. To the sample wells, add 20 µL of each respective test compound. *Note: the concentration of test compound in the 20 µL should be 5× the desired final concentration in the 100 µL 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 µL Substrate and 35 µL Assay Buffer.

   Add 40 µL Working Reagent to every well and tap plate to mix immediately.
7. Incubate the plate for 20 minutes at room temperature.
8. Add 160 µL 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 ¼ of the volumes for 96-well plate assay.

CALCULATION

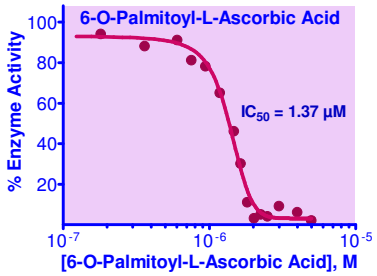
Calculate the % inhibition using the equation below:

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

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

MATERIALS REQUIRED, BUT NOT PROVIDED

Purified Human HAase (e.g. Acro Biosystems Cat # PH0-H5225) or Bovine HAase (e.g. Calzyme Cat # 091A0300) and if desired a control HAase inhibitor (e.g 6-O-Palmitoyl-L-Ascorbic Acid, TCI Cat # A0540), pipetting devices, eppendorf tubes, clear flat-bottom 96-well plates, and plate reader.



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).

LITERATURE

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.
3. Whatcott, C., et al (2011). Targeting the Tumor Microenvironment in Cancer: Why Hyaluronidase Deserves a Second Look. Cancer Discovery. 1(4):291-96.
4. Whatcott, C., et al (2016). Hyaluronidase To Enhance Nanoparticle-Based Photodynamic Tumor Therapy. Nano Lett. 16(4):2512-2521.