Hyaluronidase Inhibitor Screening Assay Kit (BA0220)

Product Datasheet | SKU BA0220

Description

Hyaluronidases are a family of enzymes that catalyse the degradation of the glycosaminoglycan hyaluronic acid, one of the major constituents of the extracellular matrix, where it contributes to both cell proliferation and migration. The role of hyaluronidases in breaking down this key factor makes them a possible target for cancer treatment. This kit uses a two-step turbidimetric reaction to measure hyaluronidase activity by the amount of hyaluronic acid hydrolysed: a stop reagent halts the enzymatic reaction and forms turbidity with residual hyaluronic acid, and the decrease in turbidity at 600 nm is directly proportional to hyaluronidase activity in the sample.

Specifications

Detection Method	Turbidimetric; read at 600 nm
Detection Range	Not specified
Sample Type	Test compounds (inhibitors); purified hyaluronidase enzyme
Species Reactivity	All
Assay Time	Approximately 30 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Store kit components at -20 degrees C upon receipt.
Shelf Life	12 months after receipt
Shipping	Room Temperature

Kit Components

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

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0220	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. This assay is based on an enzyme-catalysed kinetic reaction, so addition of Working Reagent should be quick and mixing brief but thorough; use of a multi-channel pipettor is recommended. Note that neither the enzyme hyaluronidase nor a control inhibitor is included in the kit.
2. Prior to assay, equilibrate all components to room temperature; the Working Reagent should be prepared fresh and used within two hours.
3. Prepare hyaluronidase in Enzyme Buffer and use it fresh; albumin and other proteins interfere with the assay and should not be included in the Enzyme Buffer. The protocol is optimised for human hyaluronidase (Acro Biosystems Cat # PH0-H5225) or bovine hyaluronidase (Calzyme Cat # 091A0300); dilute human hyaluronidase to 20 U/mL, or bovine hyaluronidase to 10 U/mL, in Enzyme Buffer.

4. Dissolve the test compounds (inhibitors) in a solvent of choice; DMSO at concentrations of 1 v/v% or less in the 100 microlitre enzymatic reaction will not interfere (the 20 microlitres of test compound may be in 5% DMSO).
5. For each inhibitor and inhibitor concentration being tested, transfer 40 microlitres of hyaluronidase into separate wells of a 96-well plate.
6. Transfer an additional 40 microlitres of hyaluronidase and 40 microlitres of Enzyme Buffer into separate wells for the No Inhibitor Control (NIC) and No Enzyme Control (NEC) respectively.
7. To the NIC and NEC wells, add 20 microlitres of the solvent in use with the test compounds.
8. To the sample wells, add 20 microlitres of each respective test compound; the concentration of test compound in the 20 microlitres should be 5x the desired final concentration in the 100 microlitre enzymatic reaction.
9. Incubate wells with test compounds for 15 minutes at room temperature.
10. Prepare enough Working Reagent for each well by combining 10 microlitres of Substrate and 35 microlitres of Assay Buffer, add 40 microlitres of Working Reagent to every well and tap the plate to mix immediately.
11. Incubate the plate for 20 minutes at room temperature.
12. Add 160 microlitres of Stop Reagent to each well and tap the plate to mix briefly and thoroughly.
13. Incubate for 10 minutes at room temperature and read optical density at 600 nm. This assay can be run in 384-well plates by using one quarter of the 96-well volumes.

Calculation

Calculate the % inhibition as: % Inhibition = [1 - ((OD_No Enzyme - OD_No Inhibitor) / (OD_No Enzyme - OD_Test Cpd))] x 100%, where OD_No Enzyme, OD_No Inhibitor and OD_Test Cpd are the optical density values of the No Enzyme Control, No Inhibitor Control and Test Compound respectively.

Notes

- This assay is based on an enzyme-catalysed kinetic reaction; addition of Working Reagent should be quick and mixing brief but thorough.
- Neither the enzyme hyaluronidase nor a control inhibitor is included in the kit, and the Working Reagent should be prepared fresh and used within two hours.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.