Farnesyltransferase Inhibitor Screening Kit (BA0250)

Product Datasheet | SKU BA0250

Description

A fluorimetric inhibitor screening assay for farnesyltransferase (FTase). FTase reacts with farnesyl pyrophosphate and a dansyl-peptide substrate producing fluorescence at λem/ex = 340/550 nm; inhibition is measured as a decrease in fluorescence.

Specifications

Detection Method	Fluorimetric (λex/em = 340/550 nm)
Sample Type	Farnesyltransferase enzyme and test compounds
Species Reactivity	All
Assay Time	60 minutes
Kit Size	400 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	6 months
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	12 mL	-20°C
Substrate	200 µL	-20°C
180 mM TCEP	400 µL	-20°C
10 mM Lonafarnib	50 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0250	400 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. To ensure identical incubation times, addition of Working Reagent should be quick and mixing brief but thorough; a multichannel pipettor is recommended. Note: FTase enzyme is not included in the kit.
2. Reagent preparation: use black, flat-bottom 384-well plates. Equilibrate all components to room temperature and briefly centrifuge tubes before opening. Prepare the Working Reagent (WR) fresh for each assay run. Prepare enzyme in buffer and use fresh (protocol optimised for recombinant rat FTase, Jena Bioscience cat# PR-102). Dissolve test compounds in a solvent of choice (e.g. DMSO); Lonafarnib is included as an IC50 control.
3. Enzyme and controls: transfer 5 µL of 37.2 µg/mL FTase (7.4 µg/mL final) into separate wells of a black 384-well plate. Reserve at least one well for no inhibitor (Control) and one with no enzyme (Blank); add 5 µL of 37.2 µg/mL FTase to the Control and 5 µL Assay Buffer to the Blank. Run at least duplicate reactions.
4. Add test compounds: to the enzyme wells add 5 µL of the test compounds. To the Control and Blank wells add 5 µL of the solvent used for the test compounds. Mix immediately and incubate 10 min at room temperature for test compounds to interact with FTase.

5. Prepare enough WR for all wells by mixing, per well: 0.5 µL Substrate, 20 µL Assay Buffer and 1 µL TCEP. Transfer 15 µL WR to all wells. Immediately tap the plate to mix and incubate at room temperature.
6. Read fluorescence intensity at 60 min at λex/em = 340/550 nm.

Calculation

FTase activity in the presence of test compounds: % Activity = (RFU_TestCpd – RFU_Blank) / (RFU_Control – RFU_Blank) × 100%, where RFU_TestCpd, RFU_Control and RFU_Blank are the fluorescence values of the test compound, no-inhibitor control and no-enzyme blank at 60 min.

Notes

- Normal precautions for laboratory reagents should be exercised while using the reagents.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.