EnzyFluo™ Farnesyltransferase Inhibitor Screening Kit (EIFT-400)

Fluorimetric Inhibitor Screening Assay for Farnesyltransferase

DESCRIPTION

FARNESYLTRANSFERASE (FTase, EC 2.5.1.58) catalyzes the transfer of a farnesyl group from farnesyl pyrophosphate to the cysteine residue of the C-terminus of target proteins. When not properly regulated, farnesylated proteins, including the Ras superfamily of small GTPases, can lead to developmental disorders and cancer. Simple, direct and high-throughput inhibitor screening assays find wide applications for oncology research. BioAssay Systems' EIFT-400 assay kit provides a convenient fluorimetric method to screen for potential FTase inhibitors. In this assay, FTase reacts with farnesyl pyrophosphate and a dansyl-peptide substrate with measurable fluorescence at $\lambda_{em/ex}$ = 340/550 nm. Inhibition is determined by the decrease in fluorescence.

KEY FEATURES

Safe. Non-radioactive assay.

Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

High-throughput. A Z'-factor of 0.8 and higher is routinely observed in a 384-well format. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For high-throughput screening of FTase inhibitors and evaluation of drug modulators.

KIT CONTENTS (400 TESTS IN 384-WELL PLATES)

Assay Buffer:	12 mL	**Substrate:**	200 µL
180mM TCEP:	400 µL	**10mM Lonafarnib:**	50 µL

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life: 6 months.

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.

PROCEDURES

This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation times, the addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multichannel pipettor is recommended. *Note: FTase enzyme is not included in the kit.*

Reagent Preparation: Use black, flat-bottom 384-well plates. Prior to assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The Working Reagent (WR) should be prepared fresh for each assay run.

Enzyme Preparation: Enzyme should be prepared in buffer and used fresh. The following protocol is optimized for recombinant rat FTase enzyme (Jena Bioscience, Cat #PR-102). If using a different enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well.

Test Compound Preparation: Dissolve test compounds in a solvent of choice, e.g. DMSO. It is prudent to first test the tolerance of the enzyme with the solvent of choice. In the example below, rat FTase (Jena Bioscience, Cat #PR-102) was found to tolerate up to 2v% DMSO. Lonafarnib is included as an IC_{50} control.

1. *Enzyme and Controls*. Transfer 5 µL of 37.2 µg/mL FTase (7.4 µg/mL final concentration) 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 and 5 µL of Assay Buffer to the Control and Blank wells, respectively. We recommend running at least duplicate reactions.

2. *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 that the test compounds are dissolved in. For example, if the test compounds are dissolved in 1% DMSO, add 5 µL of 1% DMSO to these wells.

   Mix *immediately* and incubate for 10 min at RT for test compounds to interact with FTase.

3. Prepare enough WR for all wells by mixing for each 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 RT.

4. Read fluorescence intensity at 60 min at $\lambda_{ex/em}$ = 340/550nm.

CALCULATION

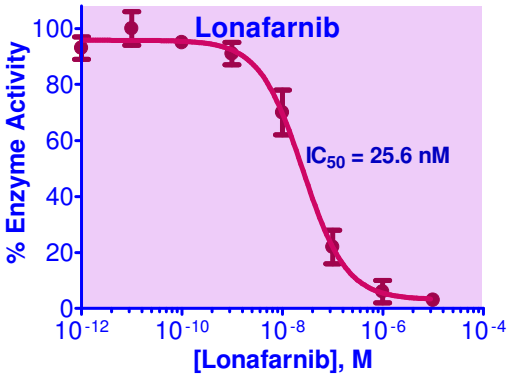
FTase activity in the presence of test compounds is calculated as follows,

$$\% \text{ Activity} = \frac{RFU_{\text{Test Cpd}} - RFU_{\text{Blank}}}{RFU_{\text{Control}} - RFU_{\text{Blank}}} \times 100\%$$

where the $RFU_{\text{Test Cpd}}$, RFU_{Control}, and RFU_{Blank} are the fluorescence values of the test compound, no inhibitor control, and no enzyme blank at 60 min.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black, flat-bottom 384-well plates (e.g. Corning™ 3573 cat# 09-761-86), centrifuge tubes and plate reader. FTase enzyme is not included.



Recombinant Rat FTase was incubated with various concentrations of Lonafarnib. Each concentration of inhibitor contained 1v% DMSO. The IC_{50} of Lonafarnib with 7.4 µg/mL FTase enzyme was determined to be 25.6 nM.

LITERATURE

[1]. Appels, Natalie et al (2005). Development of farnesyl transferase inhibitors: a review. *Oncologist*: vol. 10(8) 565-78.

[2]. Long, Stephen et al (2001). The crystal structure of human protein farnesyltransferase reveals the basis for inhibition by CaaX tetrapeptides and their mimetics: *PNAS*: vol. 98(23) 12948-12953.

[3]. Wang, Jingyuan et al (2017). New tricks for human farnesyltransferase inhibitor: cancer and beyond. *MedChemComm:* vol. 8(5) 841-854.