

Enzychrom™ Farnesyltransferase Activity Assay Kit (EFTS-400)

A Fluorimetric High-Throughput Farnesyltransferase Activity Assay

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 activity assays find wide applications for cancer research, while BioAssay Systems' EFTS-400 assay kit provides a convenient fluorimetric method for assaying FTase activity. In this assay, FTase reacts with farnesyl pyrophosphate and the dansyl-peptide substrate releasing a product that is measurable by fluorescence ($\lambda_{em/ex} = 340/550$ nm).

KEY FEATURES

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

Sensitive and accurate. Linear detection range 0.024 – 3.2 U/L FTase in a 384-well plate assay.

High-throughput. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of FTase enzyme activity in biological samples.

Kit Contents (400 tests in 384-well plates)

Assay Buffer:	12 mL	Substrate:	200 μ L
180 mM TCEP:	400 μ L		

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 time, 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 should be prepared fresh for each assay run.

Enzyme Preparation: Enzyme should be prepared in buffer and used fresh. We recommend that you experimentally determine the optimal amount of enzyme to use per well.

FTase Activity Assay in 384-well Plate

1. Transfer 5 μ L of the samples to separate wells.
2. Prepare enough Working Reagent (WR) for all sample wells by mixing for each well 0.5 μ L Substrate, 30 μ L Assay Buffer and 1 μ L TCEP. Add 25 μ L WR to all sample wells. Immediately tap plate to mix.
3. Read fluorescence intensity at time zero and at 60 min at $\lambda_{ex/em} = 340/550\text{nm}$, or read fluorescence kinetically for 60 minutes.

CALCULATION

Farnesyl transferase (FTase) activity is calculated as follows:

$$\text{FTase Activity} = \frac{(F_{60} - F_0)}{(4.3 \cdot F_0 - F_0) / [\text{Substrate}] \cdot t} \times \frac{\text{Reaction Vol } (\mu\text{L})}{\text{Sample Vol } (\mu\text{L})} \times n \text{ (U/L)}$$

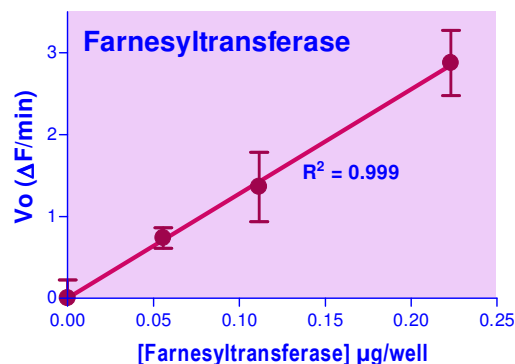
$$= \frac{F_{60} - F_0}{F_0} \times 0.303 \times n \text{ (U/L)}$$

Where F_{60} and F_0 are the measured fluorescence intensities of the samples at 60 min and 0 min, respectively. [Substrate], Reaction Vol and Samples Vol are the substrate concentration 10 μM , 30 μL and 5 μL . t is the reaction time (60 min), 4.3 is the conversion factor used to determine maximum sample fluorescence at 6 hours, and n is the sample dilution factor.

Unit definition: one unit of FTase catalyzes the transfer of 1 μmole of the farnesyl group per minute under the assay conditions.

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.



V_0 ($\Delta F/\text{min}$) versus FTase enzyme ($\mu\text{g/well}$) in the reaction.

LITERATURE

- [1]. Hougland, James et al (2010). Identification of novel peptide substrates for protein farnesyltransferase reveals two substrate classes with distinct sequence selectivities. *Journal of Molecular Biology*: 395(1), 176-190.
- [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]. Rowinsky, EK et al (1999). Ras protein farnesyltransferase: A strategic target for anticancer therapeutic development. *J Clin Oncol*: 17(11), 3631-52.

