# 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 µL |
| **150 mM DTT:** | 400 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 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 µL of the samples to separate wells.

2. Prepare enough Working Reagent (WR) for all sample wells by mixing for each well 0.5 µL Substrate, 30 µL Assay Buffer and 1 µL DTT. Add 25 µL WR to all sample wells. Immediately tap plate to mix.

3. Read fluorescence intensity at time zero and at 60 min at $\lambda_{ex/em}$ = 340/550nm, or read fluorescence kinetically for 60 minutes.

## CALCULATION

Farnesyl transferase (FTase) activity is calculated as follows:

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

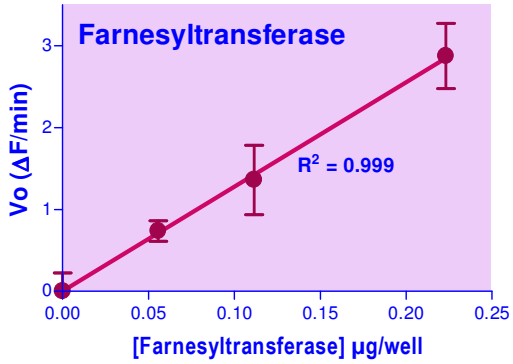
$$= \frac{F_{60} - F_0}{F_0} \times 0.303 \times n \quad \text{(U/L)}$$

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.




$V_0$ (ΔF/min) *versus* FTase enzyme (µg/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.