



ProFoldin

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INSTRUCTIONS

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Human Mitochondrial ClpP Protease Assay Kit

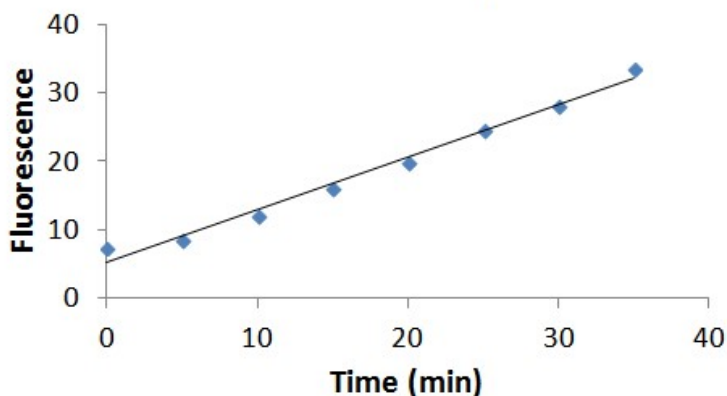
Human Mitochondrial ClpP Protease Assay Kit **Catalog No. HMP100K**

Human Mitochondrial ClpP Protease Assay Kit Plus **Catalog No. HMP100KE**

Introduction

Human mitochondrial protease ClpP has been identified as an anti-cancer drug target such as the drug target for acute myeloid leukemia (AML). The high throughput assay for human ClpP protease is based on cleavage of a labeled peptide¹ substrate that generates fluorescence at 460 nm (excitation at 380 nm). The assay can be performed in a 384-well or 96-well plate format for tests of Human ClpP protease activities and throughput screening of inhibitors for cancer therapy.

Human Mitochondrial ClpP Protease Assay



The **Human Mitochondrial ClpP Protease Assay Kit (Catalog No. HMP100K)** includes 3100 μ l of Assay Buffer and 31 μ l of 100 x Substrate and 3500 μ l of Stop solution. It is for 100 assays in 384-well plate format. All assay reagents except the enzyme are included.

The **Human Mitochondrial ClpP Protease Assay Kit Plus (Catalog No. HMP100KE)** includes 3100 μ l of Assay Buffer, 31 μ l of 100 x Substrate, 3500 μ l of Stop solution and 31 μ l of 100 x human ClpP protease. It is for 100 assays in 384-well plate format. All assay reagents are included.



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ASSAY PROTOCOL

The following assay protocol is based on the 384-well plate assay format (plate type: Matrix 4318 or alike).

The reaction volume is 30 μ l. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60 μ l.

1. Plate with inhibitors

Add 0.6 μ l of 50 x inhibitor in DMSO into each well of a 384-well plate. Use DMSO only for no-inhibitor control.

2. ClpP assay

- (1) For each 10 assay reactions, prepare a premix by mixing 275 μ l of Assay Buffer and 3.1 μ l of 100 x Human ClpP.
- (2) Add 26.4 μ l of the premix into the wells with 0.6 μ l of 50 x inhibitor or DMSO and incubate the solution at 37°C for 20 min.
- (3) Freshly dilute the 100 x Substrate with the assay buffer 10 fold to make the 10 x Substrate. Add 3 μ l of 10 x Substrate and incubate the assay reaction at 37°C.
- (4) For continuous assays, read the fluorescence emission at 460 nm with excitation at 380 nm at various time points from zero to 60 min. For endpoint assays, incubate the assay reaction solution at 37°C for 60 min, then add 30 μ l of Stop solution and read the fluorescence emission at 460 nm with excitation at 380 nm.

Assay optimization for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at <http://www.profoldin.com/services.html>.

Related Products

TBP100K	M. Tuberculosis ClpP Protease Assay Kit
TBP100KE	M. Tuberculosis ClpP Protease Assay Kit Plus
UPA1000	Ultra-sensitive High-throughput Protease Assay Kit

More information of drug targets and enzyme assays

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.