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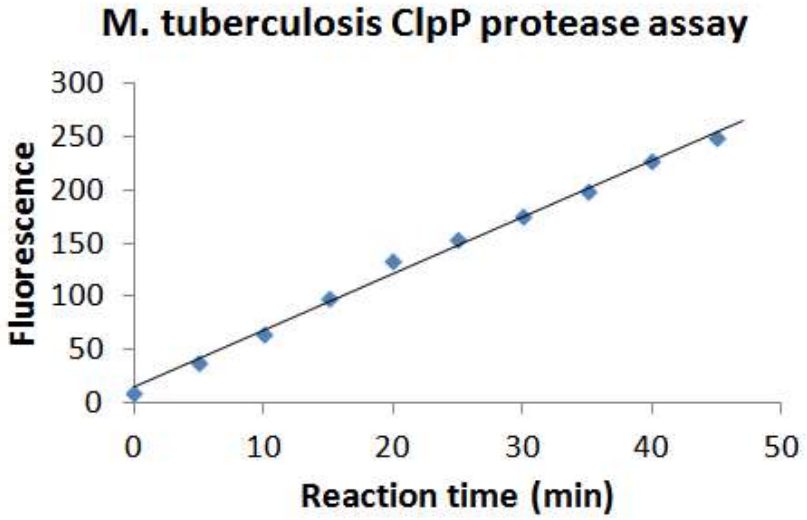
INSTRUCTIONS

ProFoldin
M. Tuberculosis ClpP Protease Assay Kit

M. Tuberculosis ClpP Protease Assay Kit Catalog No. TBP100K
M. Tuberculosis ClpP Protease Assay Kit Plus Catalog No. TBP100KE

Introduction

The disease of tuberculosis (TB) is a serious threat to global public health. It is estimated that infection of M. tuberculosis (Mtb) causes more than 1 million deaths annually. The protein degradation enzyme ClpP protease of Mtb composed of subunits P1 and P2 is a proven drug target against Mtb. ProFoldin's high throughput assay for Mtb ClpP protease is based on cleavage of a labeled peptide substrate[1] 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 Mtb ClpP protease activities and throughput screening of inhibitors against Mtb.



The **M. Tuberculosis ClpP Protease Assay Kit (Catalog No. TBP100K)** includes 3100 µl of Assay Buffer and 310 µl of 10 x Substrate and 3500 µl of Stop solution. It is for 100 assays in 384-well plate format. All assay reagents except the enzyme are included.

The **M. Tuberculosis ClpP Protease Assay Kit Plus (Catalog No. TBP100KE)** includes 3100 µl of Assay Buffer, 310 µl of 10 x Substrate, 3500 µl of Stop solution and 31 µl of 100 x Mtb ClpP1P2 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 µl. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60 µl.

1. Plate with inhibitors

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

2. Mtb ClpP assay

(1) For each 10 assay reactions, prepare a premix by mixing 275 µl of Assay Buffer and 3.1 µl of 100 x Mtb ClpP1P2.

(2) Add 26.4 µl of the prmix into the wells with 0.6 µl 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 µl of 10 x Substrate and incubate the assay reaction at 37°C.

(4) For continues 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 µl of Stop solution and read the 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

HMP100K	Human Mitochondrial ClpP Protease Assay Kit
HMP100KE	Human Mitochondrial 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.