



ProFoldin

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INSTRUCTIONS

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HIV Reverse Transcriptase Assay Kit

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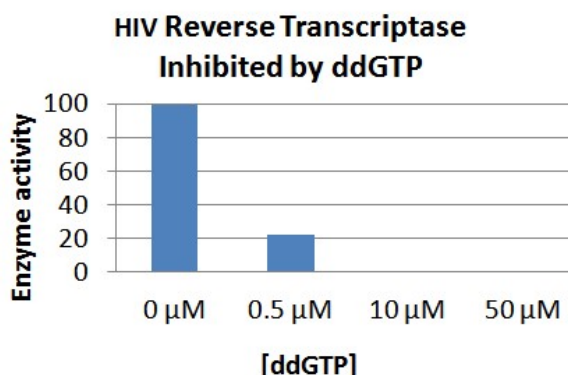
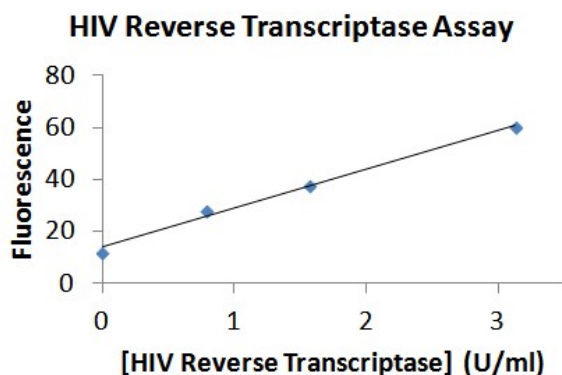
HIV Reverse Transcriptase Assay Kit Plus

Catalog No. HIV100K

Catalog No. HIV100KE

Introduction

Infection of human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS). The HIV reverse transcriptase (HIV RT) is an attractive drug target for HIV drug discovery. The HIV RT enzyme synthesizes a complementary DNA strand initiating from a primer using either RNA or single-stranded DNA as a template. The HIV Reverse Transcriptase Assay is based on measurement of the DNA molecules synthesized by HIV RT. The assay can be performed in a 384-well or 96-well plate format for tests of HIV reverse transcriptase activities and high throughput screening of inhibitors.



The **HIV Reverse Transcriptase Assay Kit** (Catalog number: HIV100K) includes 400 μ l of 10 x Buffer, 33 μ l of 100 x Template, 33 μ l of 100 x dNTPs, 1550 μ l of 2 x Dye and 1550 μ l of 50 mM EDTA. It is for 100 assays of HIV Reverse Transcriptase. The assay kit includes all reagents except the enzyme.

The **HIV Reverse Transcriptase Assay Plus** (Catalog number: HIV100KE) includes all reagents in the **HIV Reverse Transcriptase Assay Kit** (Catalog number: HIV100K) plus the enzyme, 7 μ l 500 x HIV RT.

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 and the final assay volume is 60 μ l. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60 μ l and the final assay volume is 120 μ l.

1. Reagent preparation:

(1) 10 x Template: Dilute the 100 x Template 10-fold with water. Each assay uses 3 μ l of 10 x Template.



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- (2) 10 x enzyme: Dilute the 500 x HIV RT (2 Unit / μ l) 50-fold with the 1 x assay buffer. Each assay uses 3 μ l of 10 x enzyme.
- (3) 10 x dNTP mix: Dilute the 100 x dNTP mix (1 mM dATP, 1 mM dGTP) 10-fold with water. Each assay uses 3 μ l of 10 x dNTP mix.
- (4) 1 x dye: Dilute the 2 x fluorescence dye 2-fold with 50 mM EDTA. Each assay uses 30 μ l of 1 x dye.

2. Reaction:

The total volume of each reaction mixture is 30 μ l including 18 μ l of H₂O, 3 μ l of 10 x Buffer, 3 μ l of 10 x template, 3 μ l of 10 x enzyme, 3 μ l of 10 x dNTP mix. Incubate the reaction mixture at 37°C for 60 min.

3. Detection:

Mix 30 μ l of the 1 x fluorescence dye with 30 μ l of the reaction mixture. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Assay Protocol 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

AMV100K	AMV Reverse Transcriptase Assay Kit
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
MLV100K	M-MLV Reverse Transcriptase Assay Kit
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
DPA100KH	<i>H influenzae</i> DNA Polymerase Assay Kit Plus
DPA100KN	<i>S. pneumoniae</i> DNA Polymerase Assay Kit Plus
HDPA100K	Human DNA Polymerase Alpha Assay Kit
DPB100K	Human DNA Polymerase Beta Assay Kit
DPG100K	Human DNA Polymerase Gamma Assay Kit
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
MRPA100K	Human Mitochondrial RNA Polymerase 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.