



ProFoldin

10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
FAX: (508) 845-9258 Tel: (508) 735-2539
www.profoldin.com
info@profoldin.com

INSTRUCTIONS

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Human Mitochondrial RNA Polymerase Assay Kit

Human Mitochondrial RNA Polymerase Assay Kit

Catalog No. MRPA100K

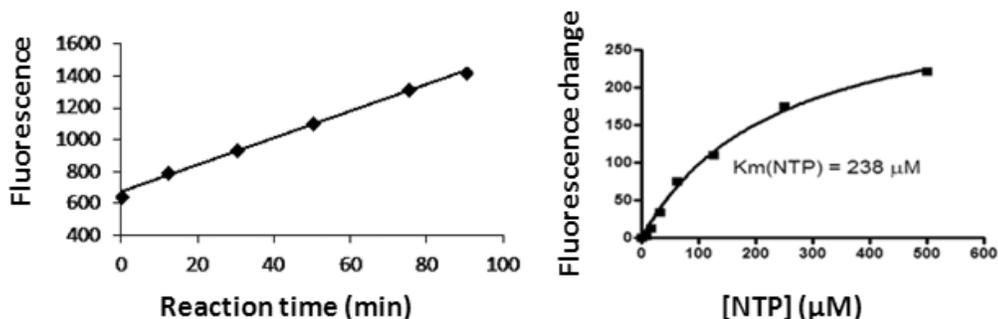
Human Mitochondrial RNA Polymerase Assay Kit Plus

Catalog No. MRPA100KE

Introduction

The human mitochondrial RNA polymerase (h-mtRNAP or POLRMT) functions as the transcriptase for expression and the primase for replication of mitochondrial DNA. It's malfunction is related to various diseases and aging. Some antiviral nucleoside analogs were reported to cause chronic toxicity and related to inhibition of mitochondrial RNA polymerase activity. The Human Mitochondrial RNA Polymerase Assay is based on measurement of the RNA molecules synthesized by the RNA polymerase using a DNA template and DNTPs. It is fluorescence assay in a 384-well or 96-well plate format. The assay can be used for measurement activities of human mitochondrial RNA polymerase and drug screens against this enzyme.

Human Mitochondrial RNA polymerase assay



The **Human Mitochondrial RNA Polymerase Assay Kit (Catalog No. MRPA100K)** includes all the assay kit components except the enzyme for 100 assays in a 384-well plate assay format: 400 μ l of 10 x Buffer, 33 μ l of 100 x DNA template, 65 μ l of 50 x NTP mix, 330 μ l of 10 x fluorescence dye.

The **Human Mitochondrial RNA Polymerase Assay Kit Plus (Catalog No. MRPA100KE)** includes all the assay kit components for 100 assays in 384-well plate assay format: 400 μ l of 10 x Buffer, 33 μ l of 100 x DNA template, 63 μ l of 50 x Human Mitochondrial RNA Polymerase, 65 μ l of 50 x NTP mix, 330 μ l of 10 x fluorescence dye.

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.



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1. Reagent preparation:

- (1) 10 x DNA: Dilute the 100 x DNA 10-fold with water. Each assay uses 3 μ l of 10 x DNA.
- (2) 10 x enzyme: Dilute the 50 x Human Mitochondrial RNA Polymerase 5-fold with the 1 x assay buffer. Each assay uses 3 μ l of 10 x enzyme.
- (3) 10 x NTP mix: Dilute the 50 x NTP mix 5-fold with water. Each assay uses 3 μ l of 10 x NTP mix.
- (4) 1 x dye: Dilute the 10 x fluorescence dye 10-fold with water. 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 DNA template, 3 μ l of 10 x enzyme, 3 μ l of 10 x NTP mix. Incubate the reaction mixture at 37°C for 2 hours.

3. Detection:

Mix 30 μ l of 1 x dye with 30 μ l of the assay reaction mixture for 5 min and read the fluorescence at 535 nm (excitation at 485 nm).

Assay Protocol for enzyme inhibition

The assay can be optimized for IC₅₀ measurement of human mitochondrial RNA polymerase. For technical support, please contact ProFoldin.

Related Products

RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
T7RPA100K	T7 RNA Polymerase Assay Kit
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
DPA100KN	<i>S. pneumoniae</i> DNA polymerase Assay Kit Plus
HDP A100K	Human DNA Polymerase Alpha Assay Kit
HDP A100KE	Human DNA Polymerase Alpha Assay Kit Plus
DPB100K	Human DNA Polymerase Beta Assay Kit
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100K	Human DNA Polymerase Gamma Assay Kit
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus
PAR100KE	Human Poly (ADP-ribose) Polymerase-1 Assay Kit Plus

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