## **INSTRUCTIONS**



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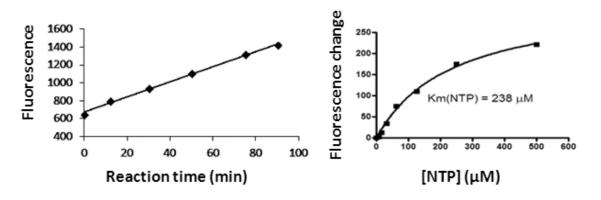
# ProFoldin Human Mitochondrial RNA Polymerase

#### Human Mitochondrial RNA Polymerase - for 100 assays

Catalog No. MRPA-100

Protein construct:	Wild-type Human Mitochondrial RNA Polymerase purified from a baculovirus
	insect protein expression system.
MW:	130 kDa
Enzyme concentration:	7.5 μΜ
RNA polymerase assay:	The RNA polymerase activity is measured by using the Human
	Mitochondrial RNA Polymerase Assay kit (Catalog No. MRPA100K).
Storage temperature:	-20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution:	Use the 1 x assay to dilute the enzyme just before the assay. Do not store
	diluted enzyme solution.

# Human Mitochondrial RNA polymerase assay



The Human Mitochondrial RNA Polymerase –for 100 assays (Catalog No. MRPA-100) includes 63 μl of 50 x Human Mitochondrial RNA Polymerase (7.5 μM). It is for 100 assays.

### Assay Protocol using the Human Mitochondrial RNA Polymerase Assay kit

The following assay protocol is based on the 384-well plate assay format (plate type: Matrix 4318 or alike). The reaction volume is 30  $\mu$ l and the final assay volume is 60  $\mu$ l. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60  $\mu$ l and the final assay volume is 120  $\mu$ 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 µl 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 µl of 10 x enzyme.
- (3) 10 x NTP mix: Dilute the 50 x NTP mix 5-fold with water. Each assay uses 3  $\mu$ l of 10 x NTP mix.
- (4) 1 x dye: Dilute the 10 x fluorescence dye 10-fold with water. Each assay uses 30  $\mu$ l of 1 x dye.

#### 2. Reaction:

The total volume of each reaction mixture is 30  $\mu$ l including 18  $\mu$ l of H<sub>2</sub>O, 3  $\mu$ l of 10 x buffer, 3  $\mu$ l of 10 x DNA template, 3  $\mu$ l of 10 x enzyme, 3  $\mu$ l of 10 x NTP mix. Incubate the reaction mixture at 37°C for 2 hours.

#### 3. Detection:

Mix 30  $\mu$ l of 1 x dye with 30  $\mu$ 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  $IC_{50}$  measurement of human mitochondrial RNA polymerase. For technical support, please contact ProFoldin.

#### **Related Products**

MRPA100KE	Human Mitochondrial RNA Polymerase Assay kit Plus
MRPA100K	Human Mitochondrial RNA Polymerase Assay kit
RPA100KE	E. coli RNA Polymerase Assay Kit Plus
RPA100KSE	S. aureus RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
DPA100KE	E. coli DNA Polymerase III Alpha Assay Kit Plus
DPA100KN	S. pneumoniae DNA polymerase Assay Kit Plus
HDPA100KE	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

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

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