



ProFoldin

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INSTRUCTIONS

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SARS-CoV-2 RNA Polymerase (RdRp) Assay Kits

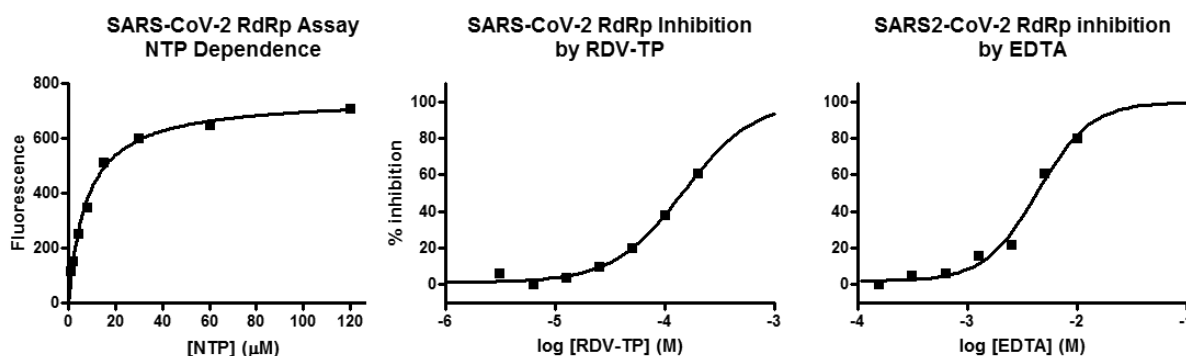
SARS-CoV-2 RNA polymerase (RdRp) assay kit-100
SARS-CoV-2 RNA polymerase (RdRp) assay kit plus -20
SARS-CoV-2 RNA polymerase (RdRp) assay kit plus -100

Catalog No. S2RPA100K
Catalog No. S2RPA020KE
Catalog No. S2RPA100KE

Introduction

The COVID-19 pandemic is caused by infection of SARS-CoV-2 or severe acute respiratory syndrome coronavirus 2, a positive-sense single-stranded RNA virus. The RNA dependent RNA polymerase (RdRp) is a key drug target for this deadly virus. The high throughput SARS-CoV-2 RdRP assay is based on detection of the RNA molecules synthesized by the RNA polymerase in the presence of an RNA template and NTPs. The assay can be performed in a 384-well or 96-well plate format for tests of the enzyme activities of the COVID-19 virus RNA polymerase (SARS-CoV-2 RdRp, the complex of nsp12, nsp7 and nsp8 proteins) and high throughput screening of inhibitors.

The gel-based assay is also available for visualization of the RNA product synthesized by RdRp and inhibition by inhibitors (<https://www.profoldin.com/covid-19-drug-targets.html>).



SARS-CoV-2 RNA polymerase (RdRp) assay kit-100 (Catalog No. S2RPA100K) includes 300 μl of 10 x Buffer, 50 μl of 50 x RNA template, 60 μl of 50 x NTPs (ATP, UTP, GTP, CTP) and 700 μl of 10 x fluorescence dye. It is for 100 assays of SARS-CoV-2 RNA polymerase in a 384-well plate format or 50 assays in a 96-well plate format. The assay kit includes all reagents except the enzyme.

SARS-CoV-2 RNA polymerase (RdRp) assay kit plus-100 (Catalog No. S2RPA100KE) includes 300 μl of 10 x Buffer, 50 μl of 50 x RNA template, 60 μl of 50 x NTPs (ATP, UTP, GTP, CTP), 700 μl of 10 x fluorescence dye and 50 μl of 50 x SARS-CoV-2 RdRp. It is for 100 assays of SARS-CoV-2 RNA polymerase in a 384-well plate format or 50 assays in a 96-well plate format.



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SARS-CoV-2 RNA polymerase (RdRp) assay kit plus-20 (Catalog No. S2RPA020KE) includes 75 µl of 10 x Buffer, 10 µl of 50 x RNA template, 12 µl of 50 x NTPs (ATP, UTP, GTP, CTP), 150 µl of 10 x fluorescence dye and 10 µl of 50 x SARS-CoV-2 RdRp. It is for 20 assays of SARS-CoV-2 RNA polymerase in a 384-well plate format or 10 assays in a 96-well plate format.

Assay Protocol for Enzyme Activity

The following assay protocol is based on the 384-well plate assay format (plate type: Matrix 4318 or alike). The reaction volume is 25 µl and the final assay volume is 90 µl. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 50 µl and the final assay volume is 180 µl.

- (1) **Reaction:** The total volume of each reaction mixture is 25 µl including 21 µl of H₂O, 2.5 µl of 10 x Buffer, 0.5 µl of 50 x template, 0.5 µl of 50 x RNA polymerase and 0.5 µl of 50 x NTPs. Incubate the reaction mixture at 37°C for 2 hours.
- (2) **Detection:** Dilute the 10x fluorescence dye with water to make the 1 x dye. Mix 65 µl of the 1 x dye with 25 µl of the reaction mixture. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm in 5 min.

Assay Protocol for Enzyme Inhibition

For the SARS-CoV-2 RdRp assay in a 96-well plate format:

- (1) Add 1 µl of compound in DMSO into each well of the 96-well assay plate.
- (2) Add 48 µl of a premix composed of 41 µl of H₂O, 5 µl of 10 x Buffer, 1 µl of 50 x Template and 1 µl of 50 x RdRp. After 5 min, add 1 µl 50 x NTP and incubate the reaction at 37 °C for 90 min.
- (3) Add 150 µl of 1x dye and read the fluorescence in 5 min.

Reference:

Pacl H. T et al, Water-soluble tocopherol derivatives inhibit SARS-CoV-2 RNA-dependent RNA polymerase. bioRxiv, Cold Spring Harbor Laboratory, July 27, 2021.

Goc A. et al, Simultaneous Inhibition of SARS-CoV-2 Infectivity by a Specific Combination of Plant-derived Compounds. European Journal of Biology and Biotechnology, Vol 2, Issue 5, p25 (2021).

Related Products – Other RNA polymerase and DNA polymerase assays

GS2RPA020KE	Gel-based SARS-CoV-2 RdRp assay kit
VRT100K	Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kit - 100
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
HDPA100KE1	Human DNA Polymerase Alpha Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus

For more information of drug targets and enzyme assays, please visit www.profoldin.com.