



ProFoldin

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INSTRUCTIONS

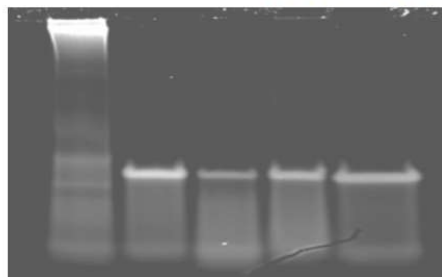
ProFoldin Gel-Based SARS-CoV-2 RdRp Assay Kit

Catalog No. **GS2RPA020KE**

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 gel-based SARS-CoV-2 RdRp assay can be used for visualization of the RNA molecules synthesized by the RNA polymerase. It can also be used to visualize the enzyme inhibition by RdRp inhibitors. High throughput fluorescence-based assay is also available for screening of RdRp inhibitors in a 384-well or 96-well plate format. For more information of the high throughput assay, please visit <https://www.profoldin.com/covid-19-drug-targets.html>.

RdRp	+	-	+	+	+
NTP	+	+	-	+	+
Inhibitor	-	-	-	RDV-TP	EDTA



The **Gel-based SARS-CoV-2 RdRp assay kit** (Catalog No. **GS2RPA020KE**) includes all the reagents for 20 samples in gel-based assays of SARS-CoV-2 RdRp activity. It includes 75 μ l of 10 x Buffer, 10 μ l of 50 x RNA template, 12 μ l of 50 x NTPs (ATP, UTP, GTP, CTP), 10 μ l of 50 x SARS-CoV-2 RdRp and 130 μ l of 5 x Gel loading buffer.

Assay Protocol for Enzyme Activity

- (1) **Reaction:** The total volume of each reaction mixture is 25 μ l including 21 μ l of H₂O, 2.5 μ l of 10 x Buffer, 0.5 μ l of 50 x template, 0.5 μ l of 50 x RNA polymerase and 0.5 μ l of 50 x NTPs. Incubate the reaction mixture at 34°C for 2 hr. At the end of the reaction, add 6 μ l of 5 x gel loading buffer.
 - (2) **Polyacrylamide gel electrophoresis:** Load 25 μ l of the sample on a 10 % native polyacrylamide gel to be run in TAE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8.3). Run the gel at 100 V until the dye frontline reaches about half way to the bottom. Stain the gel in 1x SYBR Green II solution (10,000 x SYBR Green II, Thermo Fisher Catalog No.: S7564 diluted in water 10,000 times)
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for 20 min. Visualize the RNA bands by fluorescence using same wavelength for fluorescein (Excitation at 490 ± 20 nm and emission at 520 ± 20 nm)

Assay Protocol for Enzyme Inhibition

- (1) **Reaction:** Mix 0.5 μ l of compound in DMSO with 24 μ l of a premix composed of 20.5 μ l of H₂O, 2.5 μ l of 10 x Buffer, 0.5 μ l of 50 x Template and 0.5 μ l of 50 x RdRp. Incubate the mixture for 5 min. Finally, add 0.5 μ l 50 x NTP and Incubate the reaction at 34 °C for 2 hr.
- (2) **Polyacrylamide gel electrophoresis:** Follow the same protocol described above.

Related Products

RNA polymerase assay kits:

S2RPA100K	SARS-CoV-2 RNA polymerase (RdRp) assay kit-100
S2RPA100KE	SARS-CoV-2 RNA polymerase (RdRp) assay kit plus -100
RDRP-100S2	SARS-CoV-2 RdRp complex
VRT100K	Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kit - 100
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
EGA100KE	<i>E. coli</i> DNA Primase Assay Kit Plus
HGA100KE	<i>H. influenzae</i> DNA Primase Assay Kit Plus
AGA100KE	<i>S. aureus</i> DNA Primase Assay Kit Plus
PGA100KE	<i>S. pneumoniae</i> DNA Primase Assay Kit Plus
PNP100KE	<i>E. coli</i> polynucleotide phosphorylase (PNPase) assay plus

DNA polymerase assay kits:

DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
HDP100KE	Human DNA Polymerase Alpha Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
AMV100KE	AMV 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.