Product information

Product Name: Core Direct RT-PCR Kit

Catalog No: VND500RK, VND501RK

Packing Size: 500 x 25 µl rxns and 250 x 25 µl

Shipping Condition: Ice pack / dry ice

Storage Condition: -20^{0}C

Product Description:

The Core Direct RT-PCR Kit is designed to analyze target RNA directly from blood, serum/plasma, swabs, cell cultures, bacterial colonies, and animal or plant tissue. The kit enables robust amplification without requiring prior RNA purification procedures.
The kit is optimized with our unique inhibitor-resistant, thermostable polymerases and buffer system for optimal performance. In most cases, samples can be added directly to the RT-PCR master mix without the need for pre-lysis.
The blend of polymerases is packaged separately from the 2X RT-PCR Mix, allowing scientists to optimize the enzyme amount for their specific application based on the inhibitory level in the samples. The kit ensures fast and easy preparation with a minimum of pipetting steps and is especially recommended for:

- Direct detection of viral or bacterial RNA in nasal or throat swabs
- Direct amplification from blood samples and other body fluid
- Direct PCR from cell culture
- Direct detect RNA from colony or cells
- Direct amplification of target RNA from various tissue samples
- Point-of-Care diagnostics

List of Components:

- Direct RT-PCR Pols: 2x 250 µl (500 rxns) and 1 x 250µl (250 rxns)
- 2X Direct RT-PCR Mix: 5 x 1.25 ml (500 rxns) and 3 x 1.05 ml (250 rxns)
- Lysis Buffer-B: 1 x 100 ml (500 rxns) and 1 x 50 ml (250 rxns). Please handle with care and wear personal protective equipment!

Protocol:

First Option: Directly add samples into RT-PCR mix. <u>Please always perform this option first</u>.

- For blood, serum, plasma, or other body fluid samples: Add 1-2.5 µl of the sample directly into a 25 µl RT-PCR mix.
- For cell culture: Add 1-2.5 µl of the cell culture directly into the PCR mix. Alternatively, wash the cell culture with PBS, suspend it in PBS, and add 1-2.5 µl into a 25 µl RT-PCR mix.
- For colony: Pick a single colony with a pipette tip and add it into a 25 µl RT-PCR mix.
- For nasal/throat swab samples: Transfer 100-200 µl of lysis buffer into a 1.5 ml microtube. Cut off the cotton tip with the collected nasal or throat swab and place it in the microtube. Close the tube and vortex for 15 seconds. Incubate at room temperature (20-25 °C) for 2-3 minutes. Remove the cotton tip and squeeze it out at the rim of the tube. Centrifuge briefly and transfer 1-2.5 µl of the supernatant into a 25 µl RT-PCR mix.
- For animal tissue and plant samples: Punch a 1-2 mm piece of animal tissue, plant leaf, or plant seed, and add it directly into the RT-PCR mix.

Second Option: Lyse the samples prior to adding them into PCR

Blood Samples / Liquid Samples

- Transfer 5 µl-10 µl of the blood / serum / plasma / other liquid sample into a tube containing 50-100 µl Lysis Buffer (a dilution ratio 1:10 to 1:20 in Lysis Buffer is recommended)
- Close the tube and vortex for 15 sec
- Incubate the tube at 95^{0}C for 2-3 min, then 4^{0}C for 5 min or on ice
- Centrifuge at 12,000 rpm for 2-5 min
- Transfer 1-2.5 µl of the supernatant into a 25 µl RT-PCR assay or 2-5 µl into a 50 µl RT-PCR assay

Samples from cell culture

- Collect cells and wash with PBS
- Suspend the cells in a tube with 50-100 µl Lysis Buffer
- Incubate the tube at 95^{0}C for 2-3 min, then 4^{0}C for 5 min or on ice
- Centrifuge at 12,000 rpm for 2-5 min
- Transfer 1-2.5 µl of the supernatant into a 25 µl RT-PCR assay or 2-5 µl into a 50 µl RT-PCR assay

Samples from colonies

- Transfer 5-10 µl Lysis Buffer into a 0.2 ml microtube
- Pick a single colony and add into microtube
- Incubate the tube at 95^{0}C for 2-3 min, then 4^{0}C for 5 min or on ice
- Transfer 1-2.5 µl of the supernatant into a 25 µl RT-PCR assay or 2-5 µl into a 50 µl RT-PCR assay

Samples from nasal or throat swabs

- Transfer 200 µl Lysis Buffer into a 1.5 ml microtube
- Cut off the cotton tip with the collected nasal or throat swab and place it in the microtube
- Close the tube and vortex for 15 sec
- Incubate the tube at 95^{0}C for 2-3 min, then 4^{0}C for 5 min or on ice

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- Remove the cotton tip and squeeze it out at the rim of the tube
- Centrifuge briefly and transfer 1-2.5 µl of the supernatant into a 25 µl RT-PCR assay or 2-5 µl into a 50 µl RT-PCR assay

Samples from Animal or Plant Tissue

- Prepare a small piece (1-4 mm diameter) from animal or plant tissue
- Crack plant seeds to less than 1 mm in diameter using a BeadBeater, Tissue Lyser or small hammer
- Place the sample in a microtube containing 50-100 µl Lysis Buffer
- Incubate the tube at 95^{0}C for 2-3 min, then 4^{0}C for 5 min or on ice
- Centrifuge at 12,000 rpm for 2 min
- Transfer 1-2.5 µl of the supernatant into a 25 µl RT-PCR assay or 2-5 µl into a 50 µl RT-PCR assay

Table 1. PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
De-ionized Distilled H_2O	Adjust final volume to 25 µl	
2X Direct RT-PCR Mix	12.5 µl	1X
Direct RT-PCR Pols†	1.0 µl	
Left Primer	Variable	0.2-0.5 µM
Right Primer	Variable	0.2-0.5 µM
Crude Sample*	1-2.5 µl (blood/serum/plasma/other body fluid/cells suspension/nasal or throat suspension) Single colony (from plate) 1-2 mm of piece animal or plant tissue.	
Crude Extract*	1-2.5 µl / 25 µl or 2-5 µl / 50 µl	4-10%

†We strongly recommend conducting an enzyme titration experiment by testing enzyme concentrations ranging from 0.5 to 1.0 µl per 25 µl reaction to determine the optimal enzyme concentration for your specific target.
*Please ensure that you assemble all components and distribute the master mix into each PCR tube/well before adding crude samples to the bottom of the tubes/wells. Also, avoid disturbing the bottom of the tube/well when adding samples.
Please centrifuge the samples at 12,000 rpm for 2-5 minutes to pellet debris if you are using the first option. Then, load 5-10 µl of the supernatant onto a gel to visualize the amplification product.

Table 2. Typical Cycling Parameters

A. Three steps:

RT	55-60^{0}C	20-30 min
Then denaturation	95^{0}C	5-10 min
Then followed by 35-45 cycles		
Denaturation	94^{0}C	20 to 30 sec
Annealing	50^{0}C to 68^{0}C	30 to 60 sec
Extension	70^{0}C	2 min / kb

B. Two steps:

RT	55-60^{0}C	20-30 min
Initial denaturation	95^{0}C	5-10 min
Then followed by 35-45 cycles		
Denaturation	94^{0}C	20 to 30 sec
Annealing / Extension	60-65^{0}C	2 min / kb

Note: The cycling conditions provided above serve as a guideline. We strongly recommend conducting gradient annealing temperature experiments to determine the optimal conditions for your specific targets. If you are performing multiplex RT-PCR, it is crucial to carefully design the primers and have prior knowledge of the optimal cycling conditions. We suggest conducting the RT step at 55-60°C for 20-30 minutes. You may use your own cycling conditions for the subsequent steps if you know the optimal conditions for your target. If you employ a lysis procedure, you may use a lower temperature for the RT step.

Troubleshooting guide

No PCR products	Please check your PCR settings to ensure that all necessary components are included in the PCR mix. It's also recommended to perform a gradient annealing temperature experiment to determine the optimal temperature for your specific target. This can help to improve PCR efficiency and specificity, resulting in more reliable and consistent results.
Low yield of product	If you are using the first option, it's important to set RT step at 55-60^{0}C for 20-30 minutes to ensure proper release of RNA. Additionally, you can try the following strategies to improve PCR performance: • Conduct an enzyme titration experiment to optimize the enzyme concentration for your specific target. • Increase the extension time to ensure complete amplification of the target. • Try a gradient annealing temperature experiment to identify the optimal annealing temperature for your primers. • Consider redesigning your primers if the amplification is not specific or efficient enough.
Non-specific products are observed	If you are observing non-specific products in your PCR reaction, there are several strategies you can try: • Perform a gradient annealing temperature experiment to identify the optimal temperature for your primers, which can help to reduce non-specific products. • Evaluate your primers and redesign them if necessary to improve specificity and reduce non-specific products.

This product is for R&D use only

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