Product information

Product Name: Core Direct qPCR TaqProbe Master Mix

Catalog No: VND1600TM, VND1601TM

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

Shipping Condition: Ice pack

Storage Condition: -20^{0}C

Product Description:

The Direct qPCR TaqProbe Master Mix is designed to rapid quantitative analysis of target DNA directly from blood, serum/plasma, swabs, cell cultures, bacterial colonies, and animal or plant tissue. The master mix enables robust amplification without requiring any prior DNA purification procedures.
The master mix is optimized with our unique inhibitor-resistant DNA polymerase and buffer system for optimal performance. In most cases, samples can be added directly to the PCR master mix without the need for pre-lysis.
The master mix contains all the reagents required for PCR (except template and primers) in a premixed, 2x concentrated, ready-to-use solution. It allows for fast and easy preparation with a minimum of pipetting steps and is especially recommended for:

- Direct detection of viral or bacterial DNA in nasal or throat swabs
- Direct qPCR from blood samples and other body fluid
- Direct qPCR from cell culture
- Direct colony screening quantitative analysis of target DNA from single colony
- Direct amplification of target DNA from various tissue samples
- Point-of-Care diagnostics

Please note that this kit does not contain a Passive Reference Dye ROX. If your instrument requires a reference dye, you may add ROX to the reactions. Please refer to the following table for more details.

Final concentration of ROX dye in PCR	Recommended Cyclers
500 nM	ABI® PRISM 7000,7300,7700,7900HT, 7900Fast, StepOnePlus™, StepOne™
50 nM	ABI® PRISM 7500, 7500Fast -Stratagene® Mx3000, Mx3005P, Mx4000
No	BioRad® iCycler®, iQ ™5, MyiQ™ BioRad® CFX96 Roche LightCycler® 480 MJ Research Opticon™ And Opticon™ 2, MJ Research Chromo® 4 Corbett Rotor-gene® 6000, 3000 DNA Engine Option® 2 and Chromo 4™ Eppendorf® Realplex

List of Components:

- 2X Direct qPCR Master Mix: 5 x 1.25 ml (500 rxns) and 3 x 1.05 ml (250 rxns)
- Lysis Buffer-A: 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 PCR mix. <u>Please always perform this option first</u>.

- For blood, serum, plasma, or other body fluid samples: Add 1.0 µl of the sample directly into a 25 µl PCR mix.
- For cell culture: Add 1.0 µl of the cell culture directly into the PCR mix. Alternatively, wash the cell culture with PBS, suspend it in PBS, and add 1.0 µl into a 25 µl PCR mix.
- For colony screening: Pick a single colony with a pipette tip and add it into a 25 µl 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 PCR mix.
- For animal tissue and plant samples: Use second option.

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)

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- Close the tube and vortex for 15 sec
- Incubate the tube at 98^{0}C for 5 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 PCR assay or 2-5 µl into a 50 µl 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 98^{0}C for 5 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 PCR assay or 2-5 µl into a 50 µl PCR assay

Samples from colonies

- Transfer 5-10 µl Lysis Buffer into a 0.2 ml microtube
- Pick a single colony and add into PCR mix
- Incubate the tube at 98^{0}C for 5 min, then 4^{0}C for 5 min or on ice
- Transfer 1-2.5 µl of the supernatant into a 25 µl PCR assay or 2-5 µl into a 50 µl 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 micro tube
- Close the tube and vortex for 15 sec
- Incubate the tube at 98^{0}C for 5 min, then 4^{0}C for 5 min or on ice
- 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 PCR assay or 2-5 µl into a 50 µl 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 98^{0}C for 5 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 PCR assay or 2-5 µl into a 50 µl PCR assay

PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
De-ionized Distilled H_2O	Adjust final volume to 25 µl	
2X Direct qPCR TaqProbe Master Mix	12.5 µl	1X
Left Primer	Variable	0.2-0.5 µM
Right Primer	Variable	0.2-0.5 µM
Probe#	Variable	0.2-0.5 µM
ROX (not included)	Variable (refer to the table above)	0-500 nM
Crude Sample*	1.0 µl (blood/serum/plasma/other body fluid/cells suspension/nasal or throat suspension) Single colony (from plate)	
Crude Extract*	1-2.5 µl / 25 µl or 2-5 µl / 50 µl	4-10%

#Blood samples usually need higher concentration of probe to compensate quenching effect of heme.
*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.

Typical Cycling Parameters

Initial denaturation	95^{0}C	5-15 min (for first option) 2-5 min (for second option)
Then followed by 30-45 cycles		
Denaturation	94^{0}C	20 to 30 sec
Annealing	50^{0}C to 68^{0}C	20 to 60 sec
Extension	70^{0}C	2 min / 1kb target
Final Extension	70^{0}C	5 min
Hold	4^{0}C	

Troubleshooting guide

No signal	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 signal	If you are using the first option, it's important to initially denature the samples for 5-15 minutes to ensure proper release of DNA. 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 DNA. • 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.
Unexpected variation	If you are experiencing unexpected variations in your real-time PCR results, there are a few steps you can take to address the issue. First, check your pipette to ensure that it is calibrated and accurate. Additionally, you should verify whether you need to add a ROX reference dye that is suitable for your specific PCR cycler model (please refer to the manufacturer's instructions or the provided reference table).

This product is for R&D use only

Warning: if the reagent is spilled to your skin or eyes, please wash with large amount of clean water and call emergency care immediately if necessary.

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