Product information

Product Name: Direct S/P qPCR TaqProbe Kit (No Passive Reference Dye)

Catalog No: VN800DTK, VN801DTK and VN802DTK

Packing Size: 500 x 25 µl rxns, 250 x 25 µl rxns and 100 x 25 µl rxns

Shipping Condition: Ambient temperature or ice pack

Storage Condition: -20^{0}C

Shelf life: At least 2 year if stored at -20^{0}C and 20 freezes / thaws or at least 1 week if stored at 4^{0}C for 2X Direct S/P qPCR Mix. The polymerase should always be stored at -20^{0}C and the shelf life will be at least three years from date of receipt.

Product Description:

Direct S/P qPCR TaqProbe Kit contains all the reagents needed for quickly quantitative analysis of DNA gene target from serum or plasma. This kit can be applied to direct quantitative of exogenous target, such as virus, bacteria and parasites or endogenous target, such as cfDNA, from serum or plasma. The kit is optimized with our unique inhibitor-resistant DNA polymerases for best performance. In most case, you can directly add sample into PCR master mix without pre-lysis.

This kit doesn't contain a Passive Reference Dye ROX. You may add ROX in the reactions if the instrument requires the reference dye. Please refer to the following Table for the detail.

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 S/P qPCR TaqProbe Mix: 5 x 1.25 ml (500 rxns); 3 x 1.05 ml (250 rxns); 1 x 1.25 ml (100 rxns)
- S/P qPCR Pols: 1 x 250 µl (500 rxns); 1 x 125 µl (250 rxns); 1 x 50 µl (100 rxns)
- Lysis Buffer: 1 x 10 ml (500 rxns); 4 x 1.5 ml (250 rxns); 2 x 1.5 ml (100 rxns)

Protocol:

Option A: Directly add 1.25-5 µl of serum or plasma to PCR. Please always try this option first.

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Option B: Mix 20 µl of Lysis Buffer with 5 µl of serum or plasma in PCR tube, put it in PCR cycler at 95^{0}C for 10 minutes and then cool down, centrifuge at 12,000 rpm for 5 min. The crude DNA extracts in the supernatant are ready for PCR.
If you use less or more serum or plasma, please add proportional Lysis Buffer.

PCR setting for a 25 µl reaction

Reagent	**Volume**	**Final Concentration**
2X S/P qPCR TaqProbe Mix	12.5 µl	
S/P qPCR Polymerase*	0.25-1.0 µl*	
Left Primer	variable	0.2-0.4 µM
Right Primer	variable	0.2-0.4 µM
Probe	variable	0.2-0.4 µM
Serum or Plasma / Crude Extract†	1.25-5 µl / ≤5 µl	5-20%
De-ionized Distilled H_2O	Adjust final volume to 25 µl	-

*Different sample or target may need variable amount of the Taq mutant polymerase. We strongly recommend you to do enzyme titration starting from 0.25 µl / 25 µl PCR volume to find optimal enzyme concentration for your targets and samples.
†We suggest using up to 5 µl of serum, plasma or crude extract even though our system can tolerate higher concentration of these crude samples.

Typical Cycling Parameters

Three steps:

Initial denaturation	95^{0}C	6-10 min (for Option A) 2-3 min (for Option B)
Then followed by 35-45 cycles		
Denaturation	94^{0}C	20 to 40 sec
Annealing	50^{0}C to 68^{0}C	20 to 60 sec
Plate read	Follow instrument guideline	
Extension	70^{0}C	1-2 min

Two steps:

Initial denaturation	95^{0}C	6-10 min (for Option A) 2-3 min (for Option B)
Then followed by 35-45 cycles		
Denaturation	94^{0}C	20 to 40 sec
Annealing / Extension	60^{0}C	1-2 min
Plate read	Follow instrument guideline	

Troubleshooting guide

No signal	Check your PCR setting to see if you miss some components in the PCR master mix. Try gradient annealing temperature to find optimal one for your target. Blast to check specificity of the primers and probe. Redesign them if necessary.
Low signal	Make sure to initially denature samples for 6-10 min to release DNA from the pathogens. Increase PCR cycles. Enzyme titration. Try gradient annealing temperature. Redesign primers and probe.

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

Note: This product is for R&D use only

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