

NOVADirect™ PCR Master

Cat. No. : BM0650-0125

Package : 1.25mL

Store : -20°C in a non-frost-free freezer.

Descriptions:

NOVADirect™ PCR Master is designed to perform PCR directly from biological sample such including whole blood, dried blood spot with no prior DNA extraction or sample preparation step. NOVADirect™ PCR Master is an engineered DNA polymerase which lacks both 5'→3' and 3'→5' exonuclease activities.

Recommended Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the NOVADirect™ PCR Master. For multiple reactions, scale-up volume of reaction components proportionally.

All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
3. The following table shows recommended component volumes:

Reaction Conditions

Component	25 µl reaction	50 µl reaction	Final Conc.
Direct-PCR 2X Master	12.5 µl	25 µl	1X
10µM Forward Primer	0.25~2.5 µl	0.5~5.0 µl	0.1~1.0 µM
10µM Reverse Primer	0.25~2.5 µl	0.5~5.0 µl	0.1~1.0 µM
Blood (or punched card)	0.5~2.5 µl	1.0~5.0 µl	≤ 10 %
Water, RNase-Free	up to 25 µl	up to 50 µl	NA

NOTE: The recommended starting amount is 5% blood added directly to the reaction without further modification. For blood concentrations greater than 10 %, optimization of MgCl concentration may be required.

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4. Ensure reactions are mixed thoroughly by pipetting or gentle vortex followed by a brief spin in a microcentrifuge.
5. Optional-Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.
6. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

PCR Conditions

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	30 sec.	25 ~ 40
Anneal	50~65	30 sec.	
Extend	72	1~2 min.	
Final Extension	72	5 min.	1

7. After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

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