

PhiScript Mastermix(5X)

For cDNA Synthesis in ≤ 30 minutes



PRODUCT DETAILS

Phi(ϕ)Script™ 1st strand cDNA Mastermix is extremely user friendly while making cDNA. The mastermix (5X) is uniquely blended with RNase Inhibitors, dNTPs, Mg²⁺, Reaction buffers, oligo(dT), random primers and Stabilisers. Our flagship enzyme PhiScript reverse transcriptase is provided in a separate vial. It's an engineered form of Moloney Murine Leukemia Virus reverse transcriptase (M-MuLV-RT). Point mutations are introduced in ϕ Script™ reverse transcriptase so as to reduce RNase H Activity and to improve its thermal stability.

FEATURES

1. User friendly mastermix. Just add RNA template and RT to start the reaction.
2. If RNA concentration is low, mastermix allows upto 15 μ l of template to be added (for a 20 μ l reaction mix).
3. Mastermix is freeze thaw resistant and instantly de-freezes.
4. cDNA formed can be checked with Polaris™ qPCR mastermix for real time assays.
5. cDNA Synthesis is just ≤ 30 minutes.
6. PhiScript reverse transcriptase is thermostable and cDNA Synthesis temperature can be raised to 66°C for high GC targets.

CATALOG DETAILS



Catalog details

- R6203 - 20 μ L X 50 Reactions
- R6204 - 20 μ L X 200 Reactions

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STUDY I DETAILS: EFFICIENCY

Total human RNA was isolated from skeletal cells. Exon junction primers were designed for human integrin mRNA transcript.

cDNA Synthesis conditions

Total RNA template (1 μ L=10ng), cDNA 5X mastermix(4 μ L each), ϕ Script Reverse Transcriptase, 1 μ L) and make upto 20 μ L using Nuclease Free Water.

Cycling conditions

25°C (5min)

42°C (20min)– Reverse Transcription

85°C (10min) – Inactivates RT

-20°C – Storage

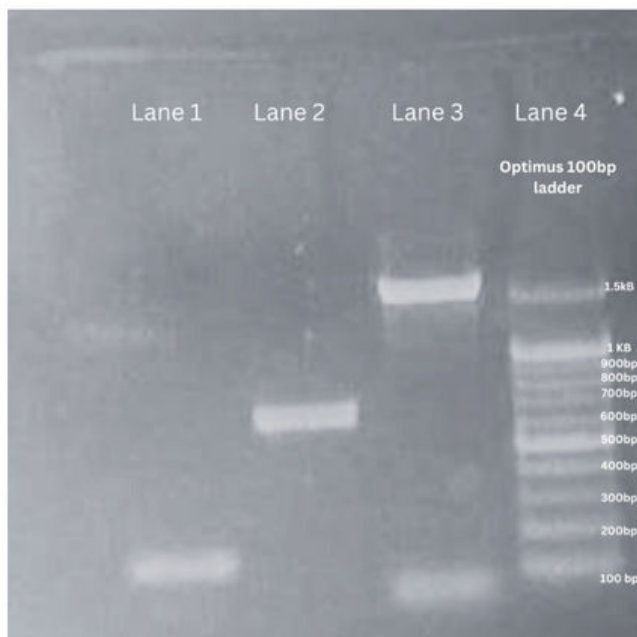


Figure (Efficiency)

(Lane 1: 150bp fragment), (Lane 2: 650bp fragment), (Lane 3: 1.6kB Fragment),
Lane 4: Optimus™ 100bp marker)

Strong band was observed for all the sizes. NTC was checked previously (not shown here) and no false positive bands were confirmed.

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STUDY II DETAILS: REVERSE TRANSCRIPTION AT VARIOUS TEMPERATURE

Total human RNA was isolated from skeletal cells. Exon junction primers were designed for human integrin mRNA transcript.

cDNA Synthesis conditions

Total RNA template (1 μ L=10ng), cDNA 5X mastermix(4 μ L each), ϕ Script Reverse Transcriptase, 1 μ L) and make upto 20 μ L using Nuclease Free Water.

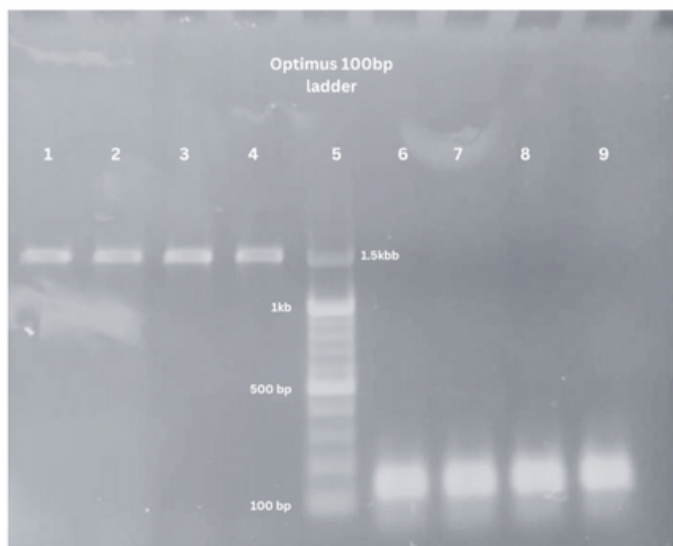
Cycling conditions

25°C (5min)

37°C (30min), 42°C (30min), 50°C (30min), 60°C (15min) – Reverse Transcription (Separate vials)

85°C (10min) – Inactivates RT

-20°C – Storage



(Lane 1: RT@37°C, 1.6kb transcript),(Lane 2: RT@42°C, 1.6kb transcript),(Lane 3: RT@50°C, 1.6kb transcript),(Lane 4: RT@60°C, 1.6kb transcript),Optimus™ 100bp marker),(Lane 6: RT@37°C, 150bp transcript),(Lane 7: RT@42°C, 150bp transcript),(Lane 8: RT@50°C, 150bp transcript),(Lane 9: RT@60°C, 150bp transcript)

Strong band was observed for all the sizes. NTC was checked previously (not shown here) and no false positive bands were confirmed.

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STUDY III DETAILS: COMPETITION COMPARISON

DX/DT cDNA 5X performance was compared against other brands in the market. Total human RNA was isolated from skeletal cells. Exon junction primers were designed for human integrin mRNA transcript.

cDNA Synthesis conditions

Total RNA template (1 μ L=10ng), cDNA 5X mastermix(4 μ L each), ϕ Script Reverse Transcriptase, 1 μ L) and make upto 20 μ L using Nuclease Free Water.

Cycling conditions

25°C (5min)

42°C (30min) – Reverse Transcription (Separate vials for competitors as per their instruction)

85°C (10min) – Inactivates RT

-20°C – Storage



Figure (Competition comparison)

(Lane 1: iScript, Biorad),
(Lane 2: Verso, Thermo),
Lane 3: TAKARA, cDNA Kit),
(Lane 4: DX/DT 5X Mastermix
and Optimus™ 1kb marker),

Performance of DX/DT Phiscript 5X mastermix is comparable to leading brands in the market.