TruScript Core H Minus RT Master Mix with DNase

FB02013005 50 Reactions
FB02013020 200 Reactions
Store at -25°C to -15°C

The TruScript Core H Minus cDNA Synthesis Master Mix offers a convenient one-tube workflow for cDNA synthesis, optimized for two-step RT-qPCR applications. The kit includes a double-strand specific DNase (dsDNase) that removes contaminating genomic DNA from RNA samples in 2 minutes without affecting RNA quality or quantity. Its specific activity towards double-stranded DNA ensures RNA and single-stranded DNA (like cDNA and primers) remain intact, allowing dsDNase-treated RNA to be directly used in reverse transcription reaction. The master mix contains TruScript Core H Minus Reverse Transcriptase (RT) and RNase Inhibitor. The RT enzyme, provides high thermostability, robustness, and increased cDNA synthesis rate. The RNase Inhibitor protects RNA from degradation at temperatures up to 55°C. The mix also contains reaction buffer, dNTPs, oligo(dT)$_{18}$, and random hexamer primers. It supports reproducible cDNA synthesis from 1 pg to 1 µg of total RNA at 42-55°C, completing the reaction in 15-30 minutes.

Kit Contents

Reagents	50 Reactions	200 Reactions
TruScript Core H Minus RT Master Mix (5X)	200 µL	800 µL
TruScript Core No RT Control	200 µL	200 µL
dsDNase	50 µL	200 µL
10X dsDNase Buffer	100 µL	400 µL
Water, nuclease-free	1.25 mL	1.25 mL

RT Reaction Setup and Protocol

1. Add the following reagents into a sterile, RNase free tube on ice in the following order:

Component	Volume	Notes
10X dsDNase Buffer	1 µL	-
dsDNase	1 µL	-
Template RNA Total RNA or Poly(A) mRNA or Specific RNA	 1 pg to 1 µg 0.1 pg to 100 ng 0.01 pg to 100 ng	Assess RNA integrity prior to cDNA synthesis
Water, nuclease-free	to 10 µL	-

2. Mix gently and centrifuge.
3. Incubate at 37°C in a preheated thermomixer or water bath for 2 minutes.
4. Chill on ice, briefly centrifuge and place on ice.
5. Add the following components to the tube on ice:

Component	Volume	Notes
TruScript Core H Minus RT Master Mix (5X)	4 µL	Contains oligo(dT)$_{18}$ and random hexamer primers to prime synthesis of first strand cDNA. This primer mixture ensures high sensitivity in low copy number transcript detection assays.
Water, nuclease-free	6 µL	-

6. Mix gently and centrifuge.
7. Optional: Incubate at 25°C for 10 minutes if random hexamer primers used.
8. Incubate at 42°C for 15 minutes. Note. If using >1 µg RNA template, increase the reaction time to 30 minutes. For RNA templates that are GC-rich or have a large amount of secondary structure, the reaction temperature can be increased to 55°C.
9. Terminate the reaction by heating at 85°C for 5 minutes.

The reaction product of the first strand cDNA synthesis can be used directly in qPCR. Store at -20°C for up to one week, or -70°C for long-term storage. Avoid freeze-thaw cycles of the cDNA.

qPCR

2 µL of the RT product is used as template for subsequent qPCR in a 25 µL total volume.

Control Reactions

Prepare negative control reactions to verify the results of the first strand cDNA synthesis. Template RNA and reverse transcription incubation conditions should reflect experimental sample conditions.

1. Add the following reagents into a sterile, RNase free tube on ice in the following order:

Component	No RT Control	No Template Control
TruScript Core H Minus RT Master Mix (5X)	-	4 µL
Template RNA	varies	-
Water, nuclease-free	to 20 µL	to 20 µL

2. Mix gently and centrifuge.
3. Optional: Incubate at 25°C for 10 minutes if random hexamer primers used.
4. Incubate at 42°C for 15 minutes.
5. Terminate the reaction by heating at 85°C for 5 minutes.