

TruScript Pro H Minus RT Master Mix

FB02010005	50 Reactions
FB02010020	200 Reactions
Store at -25°C to -15°C	

The TruScript Pro H Minus RT Master Mix offers a convenient one-tube workflow for cDNA synthesis, optimized for two-step RT-qPCR applications. It includes TruScript Pro 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 2.5 µg of total RNA at 50-60°C, completing the reaction in 15-30 minutes.

Kit Contents

Reagents	50 Reactions	200 Reactions
TruScript Pro H Minus RT Master Mix (5X)	200 µL	800 µL
TruScript Pro No RT Control	200 µL	200 µL
Water, nuclease-free	1.25 mL	1.25 mL

RT Reaction Setup and Protocol

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

Component	Volume	Notes
TruScript Pro 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.
Template RNA		RNA template must be free of DNA contamination.
Total RNA or	1 pg to 2.5 µg	
Poly(A) mRNA or	0.1 pg to 250 ng	Assess RNA integrity prior to cDNA synthesis
Specific RNA	0.01 pg to 250 ng	
Water, nuclease-free	to 20 µL	-

1. Mix gently and centrifuge.
2. Optional: Incubate at 25°C for 10 minutes if random hexamer primers used.
3. Incubate at 50°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 60°C.
4. 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 Pro 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 50°C for 15 minutes.
5. Terminate the reaction by heating at 85°C for 5 minutes.