SIGNALCHEM DIAGNOSTICS

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INSTRUCTIONS FOR USE

Product Name: Advanced Reverse Transcriptase, Glycerol-Free (600 U/µL) **Catalog # RTH07-E29B**

Denaturation of RNA Template: (Optional steps; to open the secondary structures and improve yield of cDNA)

Step 1. In an RNase free centrifuge tube add the following reaction components on ice:

1 µL	Oligo (dT) 18 (50 µM) Or Random Primers (50 µM) Or Gene Specific Primers (2 µM)
10 pg - 5 µg	RNA Template
Up to 13 µL	RNase Free ddH_2O

- For mRNA use 10 pg – 500 ng of templates, or 10 pg - 5 µg of total RNA.

Step 2. Incubate at 65°C for 5 min.

Step 3. Chill on ice for 2 min.

Step 4. Briefly vortex and centrifuge to collect the reaction and prepare the next reaction.

cDNA Synthesis Reaction:

Step 1. On ice add the following reaction components:

13 µL	Denaturation of RNA Step (Previous Reaction)
1 µL	RNase inhibitor (40 U/µL)
200 U	Reverse Transcriptase (600 U/µL)
1 µL	dNTP Mix (10 mM)
4 µL	5x Buffer

Step 2. Run the following thermal cycling settings:

Annealing	25°C	5 min
Reverse Transcription	42°C	15-30 min
Inactivation of RT	85°C	5 min

- For Oligo (dT) 18 or Gene Specific Primers the annealing step can be omitted.
- For complex secondary structures or high GC content, increase the reverse transcription temp from 42°C to 50-55°C.

Step 3. The product can be used in the next experiment, stored at -20°C for short term or -80°C for long term storage.