Synthesize First-Strand cDNA

A 20-µL reaction volume can be used for 50 ng–5 µg of total RNA.

Add the following components to a nuclease-free microcentrifuge tube.

Component	Volume
Total RNA, or miRNA	50 ng-5 µg
• **HiPure-Anchored-Oligo (dT)$_{18}$ primer (0.5 µg/µl)**	1 µl
• **HiPure-Random hexamer primer (0.1 µg/µl)**	1 µl
Or	
• **Gene Specific primer**	2 pmol
RT-PCR Grade Water	To 12 µl

1. Heat mixture to 65°C for 5 minutes
2. Quick chill on ice for 2 min.
3. Collect the contents of the tube by brief centrifugation and add:

Component	Volume
5 × RT-Buffer (specialized for RTase)	4 µl
HunteRNase-P, Recombinant (50 U/µl) *	1 µl
RTase, Recombinant Reverse transcriptase	1 µl
HiPure-dNTP mix (10 mM, 2.5 mM each)	2 µl

*For nRT-ROSET kit, please add the same amount of RT-PCR Grade Water instead of HunteRNase-P, Recombinant (50 U/µl).

4. For HiPure-Anchored-Oligo (dT)$_{18}$ primer or gene specific primer, incubate at 42°C for 30-60 min.

- **Note**: In case of using HiPure-Random hexamer primer (0.1 µg/µl), incubate at 25˚C for 10 min, then at 42˚C for 30-60 min.

5. Incubate at 85˚C for 15 s to inactive enzymes.

The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (>1 kb) may require the removal of RNA by RNase treatment.

Prepare PCR Reaction

It is very important to consider, adding larger amounts of the cDNA may not increase amplification and may result in decreasing PCR product amount.

Add the following to a PCR reaction tube for a final reaction volume of 50 µl:

Component	Volume	Final Concentration
Template	Variable	As required
Forward Primer (10 µM)	0.5 µl	0.2 µM
Reverse Primer (10 µM)	0.5 µl	0.2 µM
ROMAX, 2X (order by Cat No EB983001 and EB983002)	12.5 µl	1x
ddH2O	Up to 25 µl	-