



*Precision nanoScript*TM2 Reverse Transcription kit

Instructions for cDNA synthesis using up to 2 μ g of RNA



RT

Reverse Transcription



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Introduction

nanoScript 2 is a convenient formulation of MMLV enzyme and optimal buffer for performing the RT step within the real-time PCR work flow.

nanoScript 2 is recommended for all reverse transcription steps where the RNA added to each reaction is up to 2µg. cDNA synthesis, as measured by real-time PCR, has a linear relationship to the input RNA across this range.

RNA from a wide range of extraction protocols is suitable for reverse transcription using this kit. This includes column based methods (silica and anion exchange) as well as methods based on guanidine thiocyanate separation (TriZol®). It is important to establish the quality of the RNA before proceeding with the RT step.

nanoScript 2 uses a two-step RT process. The first of these is the 'annealing' step in which the RT primer is annealed to the denatured RNA. This reduces secondary structures in the RNA template and allows the enzyme to move freely along the template. In the second 'extension' step, the appropriate buffer is added to enable the nanoScript2 to reverse transcribe the RNA into cDNA. cDNA synthesis is initiated at the primer binding sites, creating a nascent cDNA strand whilst simultaneously degrading the original RNA template.



RT primer selection

The kit contains two different primers for the RT annealing step. This enables the kit to be used in the full range of experimental scenarios. It is acceptable to use both primers together in the same RT reaction. Alternatively, the information below can be used to select the optimum priming strategy for your experiment.

Oligo-dT primers (YELLOW)

Oligo-dT primers bind to the polyA tail of messenger RNA. This preferentially targets the RT reaction to the 3' end of the mRNA fraction. This reduces priming on the ribosomal fraction of total RNA which leads to early CT values for the target gene. Oligo-dT priming will yield a greater proportion of full length cDNAs which may be required for some downstream application e.g. cDNA library production.

Oligo-dT priming is recommended in the following experimental scenarios:

- For all standard gene quantification work
- When making a cDNA library

Random nonamer primers (RED)

It has been shown that priming with a longer random primer gives more consistent results in real-time PCR than priming with a random hexamer. The random nonamer primers supplied with this kit prime on all RNA species including ribosomal RNAs. This is a necessary strategy when there is no polyA tail on the target sequence e.g. microRNA. It is also advisable where the polyA tail may have been lost through degradation.

Random priming is recommended in the following experimental scenarios:

- When working with fragmented or partially degraded RNA e.g. from formalin fixed tissue or frozen samples.
- When quantifying different splice variants from the same gene.
- When using 18S RNA as a normalising signal.
- When measuring transcripts that do not have a polyA tail e.g. microRNA or viral RNA.
- When the real-time PCR primers are sited more than 4000bp from the polyA tail.

Gene specific primers

Gene specific priming is recognised as the highest quality method of RT priming for accurate quantitative PCR. If using a gene specific priming strategy, then neither oligo-dT nor random primers are required for the RT step. This technique however, has a major drawback in that RT specific primers will be required for all targets under investigation including housekeeping genes. This is an ideal strategy when only a small number of targets are under investigation and in difficult scenarios such as formalin fixed samples.

Gene specific primers are available as an additional product for all Primerdesign custom designed real-time PCR kits.



Kit Contents

Sufficient reagents are supplied to complete 50 Reverse Transcription reactions

- Oligo-dT primer (**YELLOW**)
- Random nonamer primer (**RED**)
- dNTP mix (10mM of each) (**ORANGE**)
- nanoScript 2 enzyme (**WHITE**)
- nanoScript 2 4X reaction buffer (**BLACK**)
- RNase/DNase free water (**WHITE**)

- (Optional) Gene specific primer, lyophilised (**GREEN**) re-suspend in 990ul Water

Reagents and Equipment to Be Supplied by User

- Thermostatically controlled “hot block” or water bath
- Pipettors and Tips
- Ice
- Vortex and centrifuge
- **RNA Template**
We recommend RNA be DNase treated prior to use to reduce the potential for real-time PCR detection of contaminating genomic DNA.
- **Thin walled 0.5ml tubes with close fitting lids**
- **Thin walled 0.2ml PCR tubes**



Storage

The Primerdesign Reverse Transcription kit should be stored at -20°C or cooler upon arrival. The shelf life for the kits is 6 months.

Primerdesign Satisfaction Guarantee

Primerdesign takes pride in the quality of all our products. Should this product fail to perform satisfactorily when used according to the protocols in this manual, Primerdesign will replace the item free of charge.

Trademarks

TriZol[®] is a registered trademark of Invitrogen Inc.

Quality Control

As part of our ISO9001 and ISO13485 quality assurance system, all Primerdesign products are monitored to ensure the highest levels of performance and reliability.



Bench-side Protocol

Annealing Step

1. For each RNA sample add the following reagents to a thin walled 0.2 ml PCR tube or equivalent.

Component	1 reaction
RNA template (up to 2µg)	X µl
RT primer (YELLOW) and/or (RED)* or (GREEN)	1.0 µl
RNAse/DNAse free water (WHITE)	X µl
Final volume	10 µl

*See Introduction for guidance on RT primer selection

2. **Apply a lid to each sample and then heat to 65°C for 5 minutes.**
This can be achieved using a thermostatically controlled “hot block” or heated water bath. Do not heat the RNA to a higher temperature as this can lead to RNA degradation.
3. **Immediately cool the tubes in an ice water bath.**
For optimal results, samples are transferred directly from 65°C to the ice. Do not allow the samples to cool prior to cooling on ice.



Extension step

1. Make up a mix according to the protocol below:

You will need 10µl for each RT reaction.

When working with 1ng - 2µg of RNA:

Component	1 reaction
nanoScript2 4X Buffer (BLACK)	5.0 µl
dNTP mix 10mM (ORANGE)	1.0 µl
RNAse/DNAse free water (WHITE)	3.0 µl
nanoScript2 enzyme (WHITE)*	1.0 µl
Final volume	10 µl

When working with < 1ng of RNA:

Component	1 reaction
nanoScript2 4X Buffer (BLACK)	5.0 µl
dNTP mix 10mM (ORANGE)	1.0 µl
RNAse/DNAse free water (WHITE)	2.5 µl
nanoScript2 enzyme (WHITE)*	1.5 µl
Final volume	10 µl

2. Add 10 µl of this mix to each of the samples on ice.

3a. For oligo-dT or gene specific priming alone (YELLOW** or **GREEN**)**

Apply a lid to each sample, mix tubes by briefly vortexing followed by a pulse spin and incubate at 42°C for 20 minutes.

3b. When using random primers alone (RED**) or in combination with oligo dT or gene specific primers**

Apply a lid to each sample, mix tubes by briefly vortexing followed by a pulse spin and incubate at 25°C (room temperature) for 5 minutes and then at 42°C for 20 minutes.

4. Heat inactivate the reaction by incubation at 75°C for 10 minutes.

5. Store cDNA samples at -20°C until use.