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RT Protocol Part

Important notes before getting started

- **Thaw One-Step cDNA RT-PCR 2X enzyme mix, 2X buffer and primer solutions** on ice.
  Keep the solutions on ice after complete thawing.
  Mix well before use to avoid localized differences in salt concentration.

Recommended protocols for cDNA synthesis:

RT-PCR assay without sample denaturation

Recommended for most standard combinations of template RNA and primers, sample denaturation can be omitted with no negative effect on results.

Add the following components to a nuclease-free microtube. Pipett on ice and mix the components by pipetting gently up and down. In general, water, RNA and primers should be mixed together before the remaining components are added.

Protocol for cDNA synthesis without prior sample denaturation (mix 1)

Component	Stock conc.	Final conc.,	50µL assay
RT-PCR 2X reaction mix (green cap):	2X	1X	25µL
RNA template	-	1ng to 500ng polyA RNA or 10ng to 5µg total RNA	x µL
forward Primer	10µM	200-400nM	1-2µL
reverse Primer	10µM	200-400nM	1-2µL
RT-PCR enzyme mix (red cap) *:	-	-	2µL
Nuclease-free water (M6340)	-	-	fill up to 50µL

* The Genaxxon RT-PCR enzyme mix already contains RNase inhibitor that is recommended and may be essential when working with low amounts of starting RNA.

Continue with reverse transcription and thermal cycling as recommended.

RT-PCR assay with sample denaturation (RNA/primer with a high degree of secondary structure)

Sample denaturation is particularly recommended for RNA targets that exhibit a high degree of secondary structure, for self- or cross-complementary primers and for initial experiments with new targets.

Preparation of the RNA Template / Primer Mix

Add the following components to a nuclease-free microtube and mix by pipetting gently up and down.

Protocol for cDNA synthesis with prior sample denaturation (mix 2)

Component	Stock conc.	Final conc.,	assay
RNA template	-	1ng to 500ng polyA RNA or 10ng to 5µg total RNA	x µL
forward Primer	10µM	200-400nM	1-2µL
reverse Primer	10µM	200-400nM	1-2µL
RNase-free water	-	-	fill up to 10µL

Denaturation and primer annealing

Incubate the mixture (mix 2) at 70°C for 5 min. and place it at room temperature for 5 min.

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Preparation of the RT-PCR mix

Add the following components to a nuclease-free PCR-tube and mix by pipetting gently up and down (mix 3)

Component	Stock conc.	Final conc.,	50µL assay
RT-PCR 2X reaction mix (green cap):	2X	1X	25µL
RT-PCR enzyme mix (red cap) *:	-	-	2µL
Nuclease-free water (M6340)	-	-	fill up to 40µL

Complete RT-PCR Mix (mix 4)

Add 40µL of RT-PCR mix (mix 3) with 10µL RNA Template / Primer mix (mix 2) to complete 50µL assays.
Pipett on ice and mix by pipetting gently up and down.

Continue with reverse transcription and thermal cycling as recommended.

Reverse transcription and thermal cycling

Place the vials of mix 1 or mix 4 in a PCR cycler and start the following program.

Step	Temperature	Incubation	Repeats
1. Reverse transcription	50°C	30-60 min.	1x
2. Initial denaturation	95°C	5 min. *	1x
3a. denaturation 3b. annealing 3c. elongation	95°C 55-65°C ** 72°C	10 sec 20 sec. 1 min/kb ***	30-40x
4. final elongation (optional)	72°C	5 min.	1x

- The optimal incubation time for the reverse transcription step depends on the length of cDNA.
- Incubation of 60 min is recommended for cDNA fragments of more than 2,000 bp length.
- The optimal temperature depends on the structural features of the RNA.
- Increase the temperature to 55 °C for difficult templates with high secondary structure.

Note: The optimal reaction time and temperature should be adjusted for each particular RNA.

* A prolonged initial denaturation time of up to 5 min. is recommended to inactivate the reverse transcriptase.
** The annealing temperature depends on the melting temperature of the primers
*** The elongation time depends on the length of the amplicon. A time of 1 min. per 1kb is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary.

Note that optimal reaction times and temperatures should be adjusted for each particular RNA / primer pair.

Related Products

Cat #	Description
M3022	100mM dUTP solution. Available in package sizes of 20µmol, 100µmol and 500µmol.
M3096	Uracil-DNA-Glycosylase. Available in package sizes of 200 units and 1000 units.

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