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One-Step cDNA RT-PCR Kit (2X)

	Cat#	M3136.0250	M3136.1250	Colour code of cap
Component				
One-Step cDNA RT-PCR 2-time master mix ***Master mix incl. all ingredients with the exception of primer and RNA template***		250 reactions (2x 1.25mL)	1250 reactions (10x 1.25mL)	**Cap with red colour code insert**
10X Buffer E complete		2x 1.2mL	5x 1.2mL	white

Product description

The Genaxxon bioscience One-Step cDNA RT-PCR 2X master mix is a ready-to-use 2-time master mix containing all reagents required for first strand cDNA synthesis and subsequent DNA amplification in one tube combining simple handling with high flexibility. The master mix is based on a genetically modified reverse transcriptase with improved thermal stability, which offers increased specificity, a high cDNA yield and improved efficiency for highly structured and long cDNA fragments.

The One-Step cDNA RT-PCR 2X master mix contains all the reagents required for the RT-PCR (except for the template and primer) to ensure quick and easy handling at a minimum of pipetting steps. The high-quality enzymes and the optimized reaction buffer with high-purity dNTPs guarantee superior PCR results.

RT-PCR is used to amplify double-stranded DNA from single-stranded RNA templates. In the RT step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template. In the first cycle of the PCR step hot start DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template.

During subsequent rounds of cycling the DNA polymerase exponentially amplifies this double-stranded DNA template. In one-step RT-PCR all components of RT and PCR are mixed in one tube so that the complete reaction can be performed without opening the tube once the reaction was started. This offers tremendous convenience when applied in routine testing and minimizes the risk of contaminations.

The One-Step cDNA RT-PCR master mix contains RNase inhibitor that is essential when working with low amounts of starting RNA.

The premium quality Reverse Transcriptase, ultrapure dNTPs and an optimized reaction buffer ensure superior results with highest reproducibility. The kit is optimized for high efficiency in a broad range of primer-template combinations.

Sensitivity:

Targets can generally be detected from <1pg to 20ng poly(A) RNA (mRNA) or 10pg to 1µg total RNA. Even lower amounts of RNA may be successfully amplified by using highly expressed transcripts.

Supplied buffers/solutions

- One-Step cDNA RT-PCR 2x conc. master mix containing reverse transcriptase, antibody-blocked hot start polymerase, RNase inhibitor, dNTPs, reaction buffer, additives and stabilizers
- PCR-grade Water

Quality Control

Nuclease activity: 50ng of radio labelled DNA or RNA is incubated with 200 units of the enzyme in 1X reaction buffer for one hour at 37°C, resulting in <1% release of free measurable radio activity in the supernatant.

Endonuclease activity: 1µg of Type 1 supercoiled plasmid DNA is incubated with 500 units of enzyme in 1X reaction buffer for one hour at 37°C. The supercoiled DNA is visualized on an ethidium bromide stained agarose gel to verify absence of nicking or cutting activity.

Purity: One-Step cDNA RT-PCR master mix is free of detectable RNase, and DNase (exo- and endonuclease) activity.

Stability and Storage

The One-Step cDNA RT-PCR 2X master mix can be stored at +2°C to +8°C for about 4 weeks but should be stored at -20°C for long term storage to keep its excellent activity.

If kept at -20°C the kit is stable for at least 12 months.

When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance.

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Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
For more information, please consult the appropriate material safety data sheets (MSDSs).
These are available online as pdf-file or on request (info@genaxxon.com).

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com).

Product Use Limitations

The Genaxxon bioscience One-Step cDNA RT-PCR 2X master mix is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

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RT Protocol Part

Important notes before getting started

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

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. Pipette 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 RT-PCR without prior sample denaturation (mix 1)

Component	Stock conc.	Final conc.,	20µL assay
One-Step cDNA RT-PCR 2X master mix	2X	1X	10µL
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
PCR-grade water	-	-	fill up to 20µL

Continue with reverse transcription and thermal cycling as recommended.

RT-PCR assay with sample denaturation

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 RT-PCR with prior sample denaturation (mix 2)

Component	Stock conc.	Final conc.,	10µL 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 at 70°C for 5 min. and place it at room temperature for 5 min.

Preparation of the RT-PCR mix after sample denaturation (mix 3)

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.,	20µL assay
RNA template/primer mix	-	-	10 µL
RNase-free water	-	-	10µL

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Reverse transcription and thermal cycling (RT-PCR) protocol

Place the vials of mix 1 or mix 3 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
M3068	One-Step cDNA RT-PCR Kit (2X).
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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