Data Sheet

IFTA AG
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Jena Bioscience

RT Master Lyophilisate

Lyophilized master mix for reverse transcription

Ready-to-use lyophilisates

	Cat. No.	Amount	Size
	PCR-158S-8TS	12 strips / 96 reactions	8-tube strips
	PCR-158L-8TS	60 strips / 480 reactions	
	PCR-158S-FTP	2 plates / 192 reactions	96-well plates (flat top, without skirt)
	PCR-158L-FTP	10 plates / 960 reactions	
	PCR-158S-HSP	2 plates / 192 reactions	96-well plates (half skirt)
	PCR-158L-HSP	10 plates / 960 reactions	

For *in vitro* use only
Quality guaranteed for 12 months
Store below 25°C
Store in an aluminium-coated bag or on a dry place
Lyophilisates may hydrate at humidity levels >70% when sealing is opened

RT Master Lyophilisate
Preloaded lyophilisates containing SCRIPT Reverse Transcriptase, dNTPs, Reaction Buffer, $MgCl_2$ and stabilizers

PCR-grade water

Description

RT Master Lyophilisate contains a genetically engineered version of M-MLV Reverse Transcriptase (M-MLV RT) with eliminated RNase H activity and increased thermal stability. The enzyme is a RNA-directed DNA polymerase that synthesizes a complementary DNA strand initiating from a primer using single-stranded RNA or DNA as template. Its enhanced thermal stability in combination with the deactivated RNase H activity results in an increased specificity, higher cDNA yield and an improved efficiency for full length cDNA synthesis compared with standard M-MLV RT. The enzyme is recommended for synthesis of cDNA from 100 bp up to 10 kb length.

Handling

RT Master Lyophilisate is delivered in reaction tube strips or 96-well plates preloaded with a complete RT master mix including dNTPs in a dry, room temperature stable format. The lyophilisate combines highest performance with convenience of use and stability. There is no need for freezing, thawing or pipetting on ice. The few remaining pipetting steps minimize the risk of errors or contaminations.
Each vial contains all components (except primers and template) required for a 20 µl reverse transcription assay. To perform the assay, only fill up the vials with a mix of primers and RNA template.

Recommended protocol for cDNA synthesis

1. Preparation of the RNA/Primer Mix
Add the following components to a nuclease-free microtube and mix by pipetting gently up and down:

Component	1 assay
RNA template	10 pg - 5 µg total RNA or 10 pg - 500 ng mRNA
primer	gene-specific primer: 10-20 ng (2-4 pmol) oligo-dT_{15-25} primer: 200-500 ng (30-75 pmol) random primer: 50-200 ng (30-120 pmol)
RNase-free water	fill up to 20 µl

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2. Denaturation and primer annealing (optional)

Please note: 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. For many standard combinations of RNA and primers heat treatment may be omitted with no negative effect on results. In general, water, RNA and primers should be mixed together before the rest of the components are added.

Incubate the mixture at 70°C for 5 min and place it at room temperature for 5 min (if using specific primer) or on ice (if using oligo-dT or random primer).

3. Dispensing the master mix

Dispense 20 µl of the RNA/Primer Mix to each lyophilisate containing tube or well of the plate.

4. Incubation

Incubate the reaction mix at 50°C for 30-60 min.

Please note: The optimal time 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 that optimal reaction time and temperature should be adjusted for each particular RNA.

5. Inactivation

Heat the mixture to 75°C for 15 min to inactivate the Reverse Transcriptase.

6. RNA removal (if required)

The cDNA can now be used as template for amplification in PCR. However, some specific DNA amplifications may requires the prior removal of RNA. Add 2 units RNase H and incubate at 37°C for 20 min.