



5×RT MasterMix

Product Number: PC58

Storage condition

Store at -20°C, valid for 12 months.

Component

Component	PC5801	PC5802
	50rxn	100rxn
5×RT MasterMix	200µl	400µl
RNase free H ₂ O	1 ml	2×1 ml

Description

The 5×RT MasterMix offer a whole component for cDNA synthesis. Which is a complete system for the efficient synthesis of first strand cDNA from RNA templates. The Mix utilizes the recombinant thermostable M-MuLV Reverse Transcriptase which exhibits lower RNase H activity than AMV reverse transcriptase.

This MasterMix already include all the reaction component. the thermostable M-MuLV Reverse Transcriptase, RNase Inhibitor, Random primers, Oligo dT Primer, dNTP Mixture, Buffer. When set up the reaction only need add water and RNA template.

Application

First Strand cDNA Synthesis. Detecting high copy and low copy genes.

Feature

1. Thermo stable MMLV, high efficiency and stable.
2. Whole component Mix, simple for reaction setup, fast reaction.
3. High concentration, much more volume for RNA template, suitable for low copy RNA template amplification.
4. The premix is not frozen under -20°C, simplified the Thaw and mixing progress.
5. Specially optimize for qPCR

Protocol

First Strand cDNA Synthesis (20µl reaction as a sample, 10µl also available)

1. Template RNA thawed on ice. RNase free H₂O thawed under RT and put on ice immediately.
2. Add the flowing component in a RNase free PCR tube.

Component	Volume
Total RNA/mRNA	≤16µl*
5×RT MasterMix	4µl (See Note 3)
RNase free H ₂ O	to 20µl

*Total RNA no more than 2µg, mRNA no more than 200ng (20µl reaction)

3. Gently mix the solution (total 20µl)
 - 3.1. If mRNA template source from eucaryotic cell (e.g., human mice cell) which has Poly(A) Tail, 42°C Incubation 15 min.
 - 3.2. If mRNA Template source from bacterial or virus prokaryotic cell, do not have Poly(A) tail, 25°C Incubation 10 min, 42°C Incubation 15 min.

Mark: If template has Complex secondary structure or GC rich can set up reaction temperature at 50°C.

4. Inactivation Reverse Transcriptase under 85°C for 5 sec.
5. The obtained cDNA products can be immediately used for qPCR reaction, or stored at -20°C and used within six months; Long

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term storage is recommended to be stored at -70°C after packaging. CDNA should avoid repeated freeze-thaw.

RT-qPCR

Take a suitable amount of cDNA products (generally not more than 1/10 of the volume of qPCR reaction) as the template of qPCR. If the expressed genes are abundant, they can be diluted according to actual conditions.

Note

1. Avoid of RNase pollution
2. To make sure reaction successful, the high-quality RNA template is suggested.
3. 5×RT MasterMix is Very viscous, and easily adsorbed on the tube and pipe tips causing loss. Pleas centrifuge shortly before use.
The enzyme in the 5×RT MasterMix are all excessive 3.6μl-3.8μl per prep will not affect the reaction.