M-MLV (RNase H-) 1st strand cDNA synthesis Kit

Cat No	Pack size
RK0100	100 reaction
RK0500	500 reaction

Description:
M-MLV cDNA Synthesis kit provides a sensitive and easy-to-use solution for two-step RT-PCR. This kit includes five tubes comprehensive of the reagents required for successful RT-PCR. The M-MLV H-reverse transcriptase is optimized for reliable cDNA synthesis over a wide dynamic range of input RNA. The enzyme is exceptionally well with a wide variety of targets.

Application:
1.First-strand cDNA synthesis for subsequent PCR or real-time PCR.
2.RT-PCR validation of gene expression data obtained from microarray experiments.
3.RT-PCR validation and quantification of gene silencing by RNA interference.

Storage conditions
-20˚C

Kit contents
M-MLV RTase H-
5x RT buffer (contain dNTP mix)
5x RNA protector
oligodT (15mer)
Random primer (6mer)

Protocol

1. Mix in the tube:
   1-5 μg of the total RNA (or 50-500 ng of polyA RNA) 10 pmole of strand-specific primer (or 250-500 ng of oligo -dT for each μg of RNA) 3 μl of 5x RNA protector add H 2 O – up to 15 μl

2. Incubate the mixture 10 min at 65˚C, stand 10-15 min at room temperature or place on ice

3. Add 1ul M-MLV RTase – 200 units
   4 ul 5x RT buffer(contain dNTP mix)
   and mix well

4. Incubate the mixture at 37- 50˚C during 30-120 min. The time of reaction depends on the length of cDNA, 30 min is for cDNA in range of 500 bp, 120 min is for cDNA more than 1..5 kb. The temperature of the reaction depends on the structural features of RNA. Use increased temperature (up to 50˚C) for the highly structured RNA.

5. Heat the mixture 10 min at 65-70˚C to inactivate the RTase.

6. Use the mixture for PCR or for other application.

For your PCR-Reaction you need 1-10 μl of your RT-PCR product.

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