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microRNA Stem Loop cDNA Kit

Product Number: PCK53

Storage condition

Store at -20°C.

Component

Component	50T	100T
5×THERMO Reaction Mix	200μL	400μL
gDNA Remover	50μL	100μL
RNase free H_2O	1.5mL	1.5mL

Description

The MicroRNA cDNA Kit for First Chain cDNA Synthesis is a specialized kit based on the principle of the stem loop method. The 5 × THERMO Reaction Mix is a tubular reverse transcription premix, containing all the reagents required for reverse transcription (THERMScript H-RTase, RNase inhibitor, dNTP Mixture, Buffer). Simply add template RNA and stem loop primers for the reaction, making cDNA synthesis more convenient and efficient. This kit uses a special gDNA Remover with DNA degradation activity, which does not require opening the lid halfway to add. With just one step, genome clearance and reverse transcription reactions can be completed simultaneously, greatly simplifying the operation steps and avoiding the risks of sample contamination and RNA degradation caused by complex sample addition processes. This product is based on THERMOscript H ⁻ RTase, which has extremely high thermal stability. Coupled with an optimized buffer system, it maximizes the synthesis of various microRNA specific reverse transcription products. The cDNA product has good compatibility and can be paired with any commercial company's fluorescence quantitative mix for downstream detection experiments.

Protocol

1. Stem loop microRNA first strand cDNA synthesis (using a 20μL reaction system as an example, a 10μL reaction system can also be used):

1.1. Thaw each component and mix each solution gently by bouncing or vortexing before use. Short centrifugation can be used to collect any remaining liquid on the tube wall to the bottom of the tube.

1.2. Add the following ingredients to the RNAse free tube: (It is recommended to prepare on ice using a PCR tube and place it in a PCR machine for reaction).

Component	Volume
Total RNA/microRNA	Up to 2μg
Stem-loop primer(2μM)	1μL
5 ×THERMO Reaction Mix	4μL
gDNA Remover	1μL
RNase free H_2O	to 20μL

*The total RNA used in the reaction must contain small molecule RNA (microRNA). This process can also use enriched microRNAs or pure microRNAs It is not possible to quantify directly with a spectrophotometer. It is recommended to add 2μL to 5μL directly. The amount of microRNA added can be determined based on the abundance of the target microRNA, but for low abundance microRNA samples (such as serum plasma extracts), a maximum volume of 8μL can be directly added..

1.3. Gently blow and mix with a pipette, and perform the first strand cDNA synthesis reaction according to the following conditions.

25°C	5 min

50°C	15 min
85°C	5sec

If the template has complex secondary structures or high GC regions, it may be helpful to increase the reaction temperature to 55°C to improve yield. The obtained cDNA product can be immediately used for qPCR reaction, or stored at -20 ° C and used within six months; Long term storage: It is recommended to pack and store at -70 ° C. CDNA should avoid repeated freeze-thaw cycles.

2. **RT-qPCR**

Take an appropriate amount of reverse transcription cDNA product (generally not exceeding 1/10 of the qPCR reaction volume) as the qPCR template, and proceed to the next step of fluorescence quantitative PCR according to the instructions of the fluorescence quantitative PCR reagent. If the expression of genes is abundant, the cDNA template can be diluted appropriately according to the actual situation.

If the stem loop primer is designed according to the recommendation of Adlai, the matching universal downstream primer sequence is 5 '- AGTGCGGGTCCGAGGTATT-3‘.

Primer design

1. Recommended sequence for universal stem loop structure:
   5 '- GTCGTACCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC -3'
2. The length of the stem loop tail complementary base (and microRNA3 'complementary base) is generally 5-8 bases. Our company recommends beginners to start with a tail complementary base of 6 bases. Reverse transcription primers only need to add 6 bases to the stem loop sequence based on the microRNA sequence.
3. Matching universal downstream primer sequence: 5 '- AGTGAGGGTCCGAGGTATT-3'
4. The stem loop primer design software can use any commercial company's design software, or you can contact Adlai to request primer design software.

Note

1. Avoid RNase contamination.
2. To ensure successful reverse transcription, it is recommended to use high-quality RNA samples.
3. 5 ×THERMO Reaction Mix and gDNA Remover contain glycerol, which is very viscous. The solution is easy to adsorb on the tube wall and the outside of the suction head, causing loss. Before use, please centrifuge and avoid the outer wall of the suction head from sticking and losing. The enzymes contained in the 5 × THERMO Reaction Mix and gDNA Remover are both excessive, even if the 5×THERMO Reaction Mix is used at a concentration of 3.6μL-3.8μL each time, and the gDNA Remover is used at a concentration of 0.8μL-0.9μL, it does not affect the effectiveness of use