Lolina A/S

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Product Information

| Product name | Lolina® V Reverse Transcriptase Fifth Generation Heat Resistant Reverse Transcriptase (Glycerol Free) |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cat.No. | NaM203020 |
| Size | 3 KU/12 KU/120 KU/300 KU/3000 KU |
| Storage and shipping | The product is shipped with ice pack. The product can be Store at 2~8°C, valid for 6 months. |
| Unit definitions | The amount of enzyme required to incorporate 1 nmol of dTTP into acid- insoluble material was defined as one unit of activity (U), using Poly(A).Oligo (dT) as template-primer, at 37° C for 10 min. |

Product description

Lolina® V Reverse Transcriptase is a new reverse transcriptase genetically engineered on the basis of M-MLV(H-)Reverse Transcriptase, which is thermally more stable than Lolina® M-MLV (H-) Reverse Transcriptase. Compared with Lolina® M-MLV(H-)Reverse Transcriptase, the enzyme is thermally stable and can withstand reaction temperatures up to 60°C, making it suitable for reverse transcription of RNA templates with complex secondary structures. Lolina® VReverse Transcriptase also has an improved ability to synthesize full-length CDNAs up to 10 kb in length, with enhanced affinity for templates and low-copy genes.

Operate

First strand CDNA synthesis procedure

1. RNA denaturation (this step is optional, RNA denaturation helps to open the secondary structure, which can greatly increase the yield of first strand cDNA).

| Components | Volume of use | |
|---------------------------------|----------------------------------------|--|
| RNase free ddH ₂ O | to 13 μL | |
| Oligo (dT)18 (50 µM) | 1 µL | |
| or Random Primers (50 µM) | or 1 µL | |
| or Gene Specific Primers (2 µM) | or 1 µL | |
| Template RNA | Total RNA: 1 ng-5 µg or mRNA: 1-500 ng | |

Heat at $65 \,^{\circ}$ C for 5 min and quickly place on ice to cool for 2 min. after briefly centrifuging to collect the reaction solution, add the reverse transcription reaction solution from the table below and gently blow to mix.

| Components | Volume of use |
|--------------------------------------------|---------------|
| Reaction solution from the previous step | 13 µL |
| 5x Lolina® V Buffer | 4 μL |
| dNTP Mix (10 mM) | 1 μL |
| Lolina® V Reverse Transcriptase (600 U/µL) | 200 U |
| RNase inhibitor (40 U/ µL) | 1 μL |
| RNase free ddH ₂ O | Το 20 μL |

2. Reverse transcription reaction system preparation (20 µL system)

3. Reverse transcription program settings

| Temperature | Time |
|---------------------|-----------|
| 25°C ^{a)} | 5 min |
| 42°C ^b) | 15-30 min |
| 85°C°) | 5 min |

a) When Random Primers are used, 25° C and incubation for 5 min are required; this step can be omitted if Oligo(dT)18 or Gene SpecificPrimers are used.

b) Reverse transcription temperature: 42° C is recommended. For high GC content templates or templates with complex secondary structures, the reverse transcription temperature can be increased to $50-55^{\circ}$ C.

c) Heat at 85°C for 5 min to inactivate reverse transcriptase.

The reverse transcription product can be used immediately for subsequent PCR or qPCR reactions, or can be stored at -20°C for short-term storage. For long-term storage, it is recommended that the product be stored at -80°C after dispensing to avoid repeated freezing and thawing.

The reverse transcriptase is also suitable for one-step RT-GPCR, it is recommended to add 10-20U reverse transcriptase for every 25 μ L of reaction system, or gradually increase the amount of reverse transcriptase according to the actual situation.

Notes

1. Please keep the experimental area clean; wear clean gloves and mask during operation; all consumables used in the experiment should be RNasefree to prevent RNase contamination.

2. All operations should be performed on ice to prevent RNA degradation.

3. To ensure high efficiency of reverse transcription, it is recommended to use high quality RNA samples.

4. This product is intended for scientific use only.

5. For your safety and health, please wear lab coat and disposable gloves.