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# MMLV Reverse Transcripase 2.0

**Product Number: RT20**

## Shipping and Storage

Store at -20℃ .

## Components

| Component | RT20 | RT20 | RT20 | RT20 |
|---|---|---|---|---|
| MMLV Reverse Transcripase2.0(200U/μl) | 10KU | 50KU | 50KU×4 | 50KU×10 |
| 5×RT Buffer | 0.4ml | 2.0ml | 2.0ml×4 | 2.0ml×10 |
| DEPC treatment of $H_2O$ | 1.0ml | 5.0ml | 20ml | 50ml |

## Description

MMLV Reverse Transcriptase 2.0 is Moloney Murine Leukemia Virus, The upgraded version of MMLV Reverse Transcriptase has RNA dependent DNA polymerase activity, DNA dependent DNA polymerase activity, and lacks RNase H activity. The DNA polymerase activity dependent on RNA can synthesize complementary DNA strands (cDNA) using RNA as a template chain; The DNA polymerase activity dependent on DNA can synthesize complementary double stranded DNA using cDNA single strands as templates. The thermal stability of Reverse Transcriptase 2.0 has been significantly improved, and it can react at 55-60℃, retaining more than 50% of enzyme activity within this temperature range. This enzyme has stronger elongation ability and can be used for longer cDNA synthesis.

Our company's MMLV Reverse Transcripase 2.0 is a recombinant enzyme expressed and purified through multiple steps.

## Concentration

200U/μl

## Features

1. No RNase H activity;
2. The length of synthesized cDNA is better than that of wild-type M-MLV reverse transcriptase;
3. Good thermal stability: can withstand reactions at 55-60℃.

## Application

1. First strand cDNA synthesis
2. Synthesis of full-length cDNA and preparation of cDNA library;
3. RT-PCR and Real Time RT-PCR reactions.

## Unit definition

At 37℃, using Poly (A) - Oligo (dT) as a template/primer, the amount of enzyme required to add 1nmol of [3H] dTTP within 10 minutes is defined as 1 active unit (U).

## Quality control

Related tests have shown that there is no contamination of exogenous endonuclease or exonuclease. PCR method for detecting residual DNA without host.

## Storage buffer

20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.01% Nonidet P40, and 50% glycerol.

**For Research Use Only**

## Note

1. It is recommended to prepare PCR reaction solution on ice before placing it into the PCR instrument for amplification. This is beneficial for improving the specificity of amplification, reducing non-specific amplification, and obtaining good PCR results.
2. To reduce the potential RNA degradation caused by RNaseA contamination, leading to cDNA synthesis failure, it is strongly recommended to add 1μl of RNase inhibitor to each reaction.
3. RNA related experiments are strictly prohibited from RNase A contamination, and disposable masks and gloves must be worn and replaced in a timely manner during operation.
4. RNA is unstable under alkaline conditions, avoid dissolving RNA in high pH solutions.

## Protocol

**(Example 1- Two step RT-PCR)**

**First strand cDNA synthesis experiment**

If the template RNA is extracted using an RNA extraction kit and residual genomic DNA has been removed, the following steps can be directly carried out; Otherwise, please remove gDNA first.

1. Add the following components to a PCR tube free of nuclease contamination:

| Component | Volume/μl | Final concentration |
|---|---|---|
| Oligo(dT)20(50μM) | | 5.0 μM |
| or Random Primers(25μM) | 1 | 2.5 μM |
| or Specific Primer(10μM) | | 1.0 μM |
| RNA Template* | 1-5 | 100ng-1μg |
| Nuclease-free Water | Up to 10 | / |
| Total | 10 | / |

**Note: The usage of Total RNA is generally 10ng~1μg; The usage of mRNA is generally 0.1ng~1μg.**

2. Incubate the above mixture at 65-70°C for 5-10 minutes, quickly remove and cool on ice for 2-3 minutes;
3. Continue to prepare the following reaction mixture:

| Component | Volume/μl | Final concentration |
|---|---|---|
| The above template RNA/primer denaturation solution | 6 | / |
| 5×RT Buffer(含 DTT) | 4 | 1× |
| dNTPs(10.0 mM) | 1 | 0.5 mM |
| MMLV Reverse Transcripase 2.0(200 U/μl) | 0.5 | / |
| RNase Inhibitor (40 U/μl)* | 1 | / |
| Nuclease-free Water | Up to 20 | / |
| Total | 20 | / |

**Note: Optional. To reduce the potential RNA degradation caused by RNaseA contamination, it is recommended to add 1μl per reaction.**

4. Gently tap and mix with your fingers, then centrifuge instantly;
5. If Oligo (dT) 20 or gene specific primers are used, react at 42-50℃ for 30-60 minutes; If using random primers, first incubate at 25℃ for 10 minutes, and then react at 42-50℃ for 30-60 minutes;
6. After the reaction is completed, hold at 80℃ for 1-5 minutes to inactivate RTase and terminate the reaction. Then cool on ice for subsequent experiments, or immediately store at -20℃.

   The obtained cDNA solution can be directly used for the synthesis of second stranded cDNA or PCR amplification, etc, It is recommended to use 1-5μl of cDNA solution during PCR amplification.

**PCR amplification of target cDNA experiment**

PCR amplification was performed using the cDNA containing the target gene obtained from the first strand cDNA synthesis experiment as a template. The following PCR reaction example is a 50μl standard PCR system, for reference only. The actual PCR

conditions should be optimized based on the template, primers, and target fragment size to determine the optimal reaction conditions.

1. Prepare PCR mixture according to the following formula

| Component | Volume/μl | Final concentration |
|---|---|---|
| 10×Taq Buffer | 5 | 1× |
| dNTPs(2.0 mM) | 5 | 0.2 mM |
| Primer F(10μM) | 1.5 | 0.3 μM |
| Primer R(10μM) | 1.5 | 0.3 μM |
| The above cDNA solution* | 1-4 | / |
| Taq(2.5U/μl) | 1.0 | 2.5U |
| dd$H_2O$ | up to 50 | / |
| Total | 50 | / |

**Note: The amount of cDNA template used in a 50μl PCR system is 10ng-1μg.**

2. PCR reaction conditions

| Temperature | Time | Cycles |
|---|---|---|
| 95℃ | 5 min | |
| 95℃ | 15 sec | 30 Cycles |
| 55~72℃ | 20 sec | |
| 72℃ | 1-2kb/min | |
| 72℃ | 1-5 min | |

## Application Example 2 One Step RT-PCR

One step RT-PCR combines RNA reverse transcription into cDNA and PCR reaction of target cDNA.

1. The following is a one-step amplification standard system (50μl).

| Component | Volume/μl | Final concentration |
|---|---|---|
| 5×RT Buffer | 10 | 1× |
| dNTPs(2.0 mM) | 5 | 0.2 mM |
| Primer F(10μM) | 2 | 0.3 μM |
| Primer R(10μM) | 2 | 0.3 μM |
| Total RNA* | X* | / |
| Taq(2.5U/μl) | 1.0 | 2.5U |
| MMLV Reverse Transcripase 2.0(200U/μl) | 0.5 | 100U |
| RNase Inhibitor (40U/μl) | 1 | 40U |
| Nuclease-free ddH2O | Zμl | / |
| Total | 50 | / |

**Note: The usage of Total RNA is generally 10 ng~1μg; The usage of mRNA is generally 1ng~1μg**

2. PCR reaction conditions

| Temperature | Time | Cycles |
|---|---|---|
| 42-50℃ | 30 min | |
| 95℃ | 3 min | |
| 95℃ | 15 sec | 30 Cycles |
| 55~72℃ | 20 sec | |
| 72℃ | 1-2kb/min | |
| 72℃ | 3 min | |