HiFi-MMLV cDNA Kit

HiFi-MMLV cDNA First Strand Synthesis Kit

Item No. H665693 (100 rxn)
Storage condition: -20℃

Product content

individual parts making up a compound	H665693 100 rxn
HiFi-MMLV, 200 U/μl	100 μl
5×RT Buffer	500 μl
Primer Mix	240 μl
dNTP Mix, 2.5 mM Each	500 μl
DTT, 0.1 M	240 μl
RNase-Free Water	1 ml

Product Introduction

This is a cDNA first strand synthesis kit designed for the first step of two-step RT-PCR. It contains all the necessary reagents for reverse transcription from RNA template to cDNA first strand, including HiFi-MMLV reverse transcriptase, reaction buffer, primers, dNTP and so on. The mutated HiFi-MMLV reverse transcriptase has no RNase H activity, which reduces the degradation of RNA in the reverse transcription reaction and makes it easier to obtain full-length cDNA. HiFi-MMLV reverse transcriptase is thermally stable, and can produce high yields of cDNA, which makes it simple and convenient to use. The system is highly compatible with subsequent PCR and quantitative PCR experiments, and can be used with a variety of PCR DNA polymerases.

Product Features

-RNase H-: mutated HiFi-MMLV reverse transcriptase with deletion of RNase H activity for easier access to full-length cDNA.
-Easy to use: the kit contains all the reagents required for reverse transcription except for the RNA template.

Caveat

1. RNase contamination should be avoided during operation to prevent RNA degradation or cross-contamination in the experiment. It is recommended that RNA operation be carried out in a special area, with special instruments and consumables, and that operators wear masks and disposable gloves and change gloves frequently.
2. Use disposable plastic containers as much as possible for the experiment. If glassware is used, it should be treated with 0.1% DEPC (diethyl ether pyrocarbonate) aqueous solution at 37℃ for 12 hours and autoclaved at 120℃ for 30 minutes before use, or the glassware should be sterilized by dry heat at 180℃ for 60 minutes before use. Sterile water used in experiments should be treated with 0.1% DEPC and autoclaved.
3. All reagents in this kit should be mixed gently, upside down, to avoid foaming and centrifuged briefly before use. The enzymes should be returned to -20℃ as soon as possible after use to avoid repeated freezing and thawing.
4. If the amount of starting RNA is less than 50 ng, it is recommended to add RNAase inhibitor (RNasin). This kit is not supplied.

Usage

Note: 10 ng-5 μg of total RNA can create a 20 μl reaction system, if the amount of total RNA is greater than 5 μg, please expand the reaction system proportionally.
i Reverse transcription procedure:
1. Dissolve RNA template, primers, dNTP Mix, DTT, RT Buffer, HiFi-MMLV and RNase-Free Water and set aside on ice.
2. Prepare the reaction system according to the following table in a total volume of 20 μl.

reagents	20 μl reaction system	final concentration
dNTP Mix, 2.5 mM Each	4 μl	500 μM Each
Primer Mix	2 μl	/
RNA Template	X μl	1 ng-5 μg
5×RT Buffer	4 μl	1×

DTT, 0.1 M	2 μl	10 mM
HiFi-MMLV, 200 U/μl	1 μl	/
RNase-Free Water	up to 20 μl	/

Attention:

1) If the amount of starting RNA is less than 50 ng, it is recommended to add RNAase inhibitor (RNasin). It is not provided in this kit.

(2) Primer Mix was prepared from Oligo (dT) and Random Primer.

3. Mix by vortex shaking and centrifuge briefly so that the solution on the walls of the tube collects at the bottom.

4. Incubate at 42° C for 30-50 minutes and at 85° C for 5 minutes. At the end of the reaction, centrifuge briefly and place on ice to cool.

5. The reverse transcription product can be used directly in PCR and fluorescence quantitative PCR reactions, or placed at -20℃ for long-term storage.

ii The following steps are recommended if reverse transcription efficiency is low, or if the RNA template secondary structure is complex and GC content is high:

1. Dissolve RNA template, primers, dNTP Mix, DTT, RT Buffer, HiFi-MMLV and RNase-Free Water and set aside on ice.

2. Prepare the reaction system according to the following table in a total volume of 13 μl .

reagents	20 μl reaction system	final concentration
dNTP Mix, 2.5 mM Each	4 μl	500 μM Each
Primer Mix	2 μl	/
RNA Template	X μl	1 ng-5 μg
RNase-Free Water	up to 13 μl	/

3. Incubate at 70° C for 10 minutes and rapidly ice bath for 2 minutes.

4. Centrifuge briefly so that the solution on the wall of the tube collects at the bottom of the tube.

5. Continue to add the following reagents to the above reaction solution:

reagents	20 μl reaction system	final concentration
5×RT Buffer	4 μl	1×
DTT, 0.1 M	2 μl	10 mM

HiFi-MMLV, 200 U/μl	1 μl	/

Caution:

1) If the amount of starting RNA is less than 50 ng, it is recommended to add RNAase inhibitor (RNasin). It is not provided in this kit.

2) Primer Mix is prepared from Oligo (dT) and Random primer.

6. Blow the mix gently and incubate at 42° C for 50 minutes and 85° C for 5 minutes.

7. At the end of the reaction, centrifuge briefly and place on ice to cool.

8. The reverse transcription product can be used directly in PCR reaction and fluorescence quantitative PCR reaction, or placed in -20℃ for long-term storage.