# ReverTra Ace Set

| | |
|---|---|
| Code No. | PU-TRT-100 |
| Lot No. | 02030D1 14MA |
| Storage | Stored at -20℃ |
| Size | 100 rxn. |

## List of components

| | | PU-TRT-100 |
|---|---|---|
| 1 | ReverTra Ace (100 U/ μl) | 1 x 10,000 units |
| 2 | 5 x RT Buffer | 1 x 1 ml |
| 3 | RNase Inhibitor (40 U/ μl) | 1 x 2,500 units |
| 4 | dNTPs Mixture (10mM each) | 1 x 500 μl |
| 5 | Oligo $(dT)_{20}$ (10 pmol/ μl) | 1 x 150 μl |
| 6 | Random Primer (25 pmol/ μl) | 1 x 150 μl |

# ReverTra Ace

The RT-PCR method, combining an RT (Reverse Transcription) reaction and a PCR (Polymerase Chain Reaction), is relatively quick and easy and enables the detection of RNA from multiple samples. This common method has become widely used in recent years to aid in RNA analysis such as, for example, in determining the amount of mRNA expression as well as abnormalities in RNA lengths. It is also used as a means for cloning cDNA. We have applied genetic engineering techniques to alter MMLV (Molony Murine Leukemia Virus) derived reverse transcriptase, eliminated RNase H activity, which constricts the combination of long-chain cDNA, and succeeded in the development of the improved enzyme ReverTra Ace, which greatly enhances the cDNA synthesis activity.

This product possesses the following features：

**1.Highly efficient cDNA synthesis**

The ReverTra Ace is an improved enzyme that greatly enhances cDNA synthesis activity. It verifies cDNA synthesis at 14Kb or above.

**2.High detection sensitivity**

Because the ReverTra Ace-α-optimizes without inhibiting the PCR reaction can be performed within the same tube as an RT reaction utilizing any kind of DNA polymerase.

**Reverse transcription reaction**

| Reagent | | Amount |
|---|---|---|
| RNase Free $H_2O$ | | (11.75-X) μl |
| 5 x RT Buffer | | 4 μl |
| dNTP Mixture (10 mM each) | | 2 μl |
| RNase Inhibitor (40U/ μl) | | 0.25 μl |
| Primer | | 1 μl from either of the following |
| Random primer* | (25 pmol/ μl) | |
| Oligo(dT)20 | (10 pmol/ μl) | |
| Sequence specific downstream primer | (10 pmol/ μl) | |
| RNA | | X μl from either of the following |
| Total RNA | : 0.1~1μg | |
| mRNA | : 50~500 ng | |
| ReverTra Ace | | 1 μl** |
| Total Volume | | 20 μl |

↓ (30℃、10min.)*
↓ 42℃、20min.
↓ 99℃、5mn.
↓ 4℃、5min.
↓ spindown

**【Note】**

* In the event of using a Random Primer, pre-incubate at 30℃ for 10 minutes in order to have sufficient annealing.

** Because the reverse transcriptase combines with the cDNA after the reaction, per from a 5 minute heat treatment at 99℃. However, adding more than necessary will render the heat treatment insufficient and may inhibit the PCR reaction. Please exercise caution.

## Common cautionary items when handing RNA

### 【1】Control the contamination of RNase

With the RT-PCR method，it is important to control the action of RNase. For this reason, along with avoiding the contamination of RNase from instrument and reagent, it is important to obtain an RNA sample of high purity. In addition, while being cautious of the environment under which the experiment is being conducted, it is recommended to wear a mask and gloves in order to avoid the introduction of RNase into the body via saliva and perspiration.

### 【2】Devices and instrument

The instrument to be used in the experiment should as mush as possible be autoclave sterilized plastic disposable types. When using glass instruments, dry-heat sterilize them or immerse them in 0.1 % Diethylpyrocarbonate (DEPC) solution at 37℃ for 12hours and then autoclave them (121℃ for 30minutes).

**In the event the amplified band cannot be verified or the amplification efficiency is unfavorable**

| Cause | Remedy |
|---|---|
| Template RNA<br>・Poor purity | ・Prepare again<br>・Increase PCR cycles |
| ・Insufficient template amount | ・Increase template amount |
| ・Deteriorating | ・Prepare again |
| ・High-order structure present | ・For repetitive usage，dispense a small amount at a time in advance<br>・Use a Random Primer for the RT<br>・Set RT reaction solutions mixed with substance other than enzyme at 65℃ for 5 minute，the RT will occur after 5 minute on ice. |