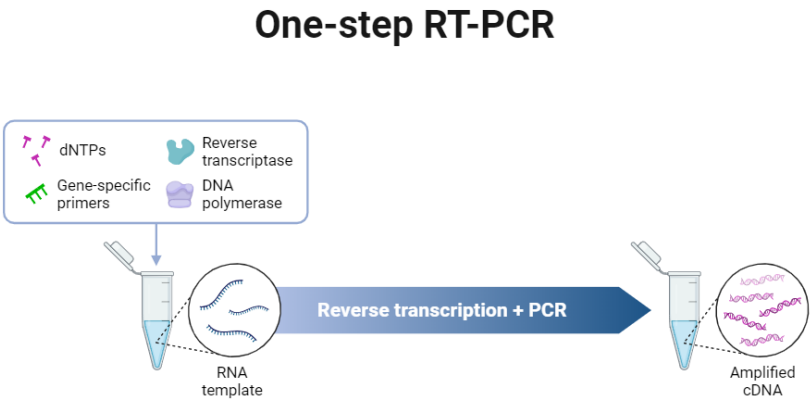
x-VITA™ One-Step RT-PCR Mix (2X)

RPCR-K01-050

Description

The 2X One Step RT-PCR Mix facilitates seamless integration of one-step reverse transcription and endpoint PCR procedures for RNA targets using any gene-specific primers (GSP). Engineered with meticulously optimized buffer components, this master mix streamlines both reverse transcription and amplification within a single reaction tube. Supplied at a 2X concentration, it simplifies usage by necessitating only the addition of GSP and template RNA, eliminating the need for additional cap opening and pipetting steps. This not only saves time but also significantly diminishes the likelihood of contamination.



Applications

- ✓ High-fidelity PCR and primer-extension reactions
- ✓ High fidelity PCR for cloning into blunt ended vectors
- ✓ Site-directed mutagenesis

Components

Component	50 rxn (50 µl/rxn)
2X One Step RT-PCR Mix *	1.25 ml
RNase-free ddH_2O	1 ml

* Contains Reverse Transcriptase, RNase inhibitor, Hotstart Taq DNA Polymerase, dNTPs and buffer

Storage

Store at -20 ºC.

Product use limitation

This product is developed, designed, and sold exclusively for research purposes and use. The product is not intended for diagnostics or drug development, nor is it suitable for administration to humans or animals.

Protocol Example

1. Preparation of reaction solution

1.1 Prepare the reaction system as follows.

1.2 Melt all reagents on ice. When preparing multiple reaction wells, leave a 10% margin for each component to avoid pipetting loss.

Component	**Volume**	**Final Conc.**
2X One Step RT-PCR Mix	25 µl	1X
Forward Primer (10 µM)	2 µl	0.4 µM
Reverse Primer (10 µM)	2 µl	0.4 µM
Template RNA	Variable	1 pg-1 µg
RNase-free ddH_2O	Variable	-
Total volume	50 µl	-

1.3 After the reaction system is ready, fully flip and mix well, and centrifuge briefly.

2. Perform RT-PCR

Fast RT-PCR mode (sequences ≤ 2 kb):

Step	**Stage**	**Cycle**	**Temperature**	**Time**
Reverse transcription	1	1	50°C [1]	30 min
Initial denaturation	2	1	95°C	2 min
Denaturation	3	30~35	95°C	30 sec
Annealing			55~68°C [2]	30 sec
Extension			72°C	Set the time by the speed of 0.5 min/kb
Final extension	4	1	72°C	5~10 min

Standard RT-PCR mode (sequences > 2 kb):

Step	Stage	Cycle	Temperature	Time
Reverse transcription	1	1	50°C [1]	30 min
Initial denaturation	2	1	95°C	2 min
Denaturation	3	30~35	95°C	30 sec
Annealing			55~68°C [2]	60 sec
Extension			72°C	Set the time by the speed of 1 min/kb
Final extension	4	1	72°C	5~10 min

1. The temperature of the reverse transcription reaction can be adjusted between 48°C and 55°C. For templates with complex secondary structures or high GC regions, increasing the reverse transcription temperature to 55ºC is conducive to improving amplification efficiency and sensitivity.

2. The denaturation temperature is set to around the primer Tm-5℃.

3. Analyse the results

Detect and analyse the reaction products by agarose gel electrophoresis.

Notes

- ✓ During the operation, pay attention to prevent RNase contamination, wear a clean mask and gloves, and use consumables that are RNase-free.
- ✓ Use high-quality RNA as a template. The presence of degraded RNA, RNase and other impurities will affect the efficiency of reverse transcription.
- ✓ The primer length should be designed between 18-30 bases and the GC content should be between 40 and 60%.