CrystalMix™ RT-PCR

• W8201 **96 rxn**

DESCRIPTION

CrystalMix™ RT-PCR kit contained all the reagents necessary for successful one-step RT-PCR in a convenient individually aliquot and freeze-dried format in an 8-strip PCR tube. The CrystalMix™ RT-PCR kit is an optimized, ready-to-use RT-PCR premixture including enhancers, stabilizers, and ambient temperature stable format. There is no need for freezing, thawing steps, or pipetting on ice, so minimized the risk of human errors and contaminations. CrystalMix™ RT-PCR kit produces fast, highly-specific, and sensitive one-step RT-PCR, from either total RNA or mRNA using gene-specific primers. An enhanced buffer allows for RT reaction temperatures up to 50°C. This can improve the detection of more difficult targets as higher RT temperatures reduce nonspecific priming and facilitate the melting of RNA secondary structures.

In addition, the CrystalMix™ RT-PCR kit includes the hot-start Taq DNA polymerase, which has been shown to improve the sensitivity and specificity for certain targets.

RT-PCR converts and amplifies a single-stranded RNA template yielding a double-stranded DNA product. In the RT step, reverse transcriptase synthesizes single-stranded DNA molecules complementary to the RNA template (first-strand cDNA). During the PCR step, a thermostable DNA polymerase first synthesizes second-strand DNA complementary to the first-strand cDNA molecules. This generates a double-stranded DNA template which is exponentially amplified in subsequent rounds of thermal cycling. RT-PCR reactions can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

APPLICATIONS

- Routine and one-step RT-PCR amplification of RNA templates
- Multiple band detection or genotyping

STORAGE CONDITIONS

- Store at below 25°C in the airtight pouch with the desiccant.
- Once opened, completely reseal the pouch with zipper.
- In high humidity environments, store unopened and resealed pouches in a desiccator to maximize product lifetime.
- Do not use once the cone-shape mix shrinks as dot-form. It damaged by re-hydration.

NOTE

Do not contaminate the CrystalMix™ RT-PCR kit with primers and template RNA used in individual reactions.

QUALITY CONTROL

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Quality Authorized by : Jamie Ahn

PROTOCOL

Please read through the entire protocol before starting.
Use the required number of tubes and immediately put the remaining tubes in the pouch and seal with the zipper.

1. Place the RT-PCR tablet mix tube on the PCR tube rack.
2. Add the reaction component to the RT-PCR tablet mix tube as the following table shows recommended component volumes:

REACTION CONDITIONS

Component	20 µl reaction	Final Conc.
CrystalMix™ RT-PCR tube	1 tube	1X
10µM Forward Primer	0.2~2.0 µl	0.1~1.0 µM
10µM Reverse Primer	0.2~2.0 µl	0.1~1.0 µM
Template RNA	≥ 1 µl	as needed
Water, RNase-Free	up to 20 µl	NA

NOTE: In general, use greater than 0.5 µM primers for sensitivity and less than 0.5 µM for specificity.

3. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
   (Optional) Overlay reactions with one-half volume PCR-grade mineral oil when not using a heated lid on a thermal cycler.
4. Transfer tubes on ice into a thermal cycler pre-warmed at the reverse transcription temperature for the best results. The following table shows recommended cycling conditions:

ONE-STEP RT-PCR CONDITIONS

Step	Temp (°C)	Time	Cycle
Reverse Transcription	42~ 55	10 ~30 min.	1
Initial Denaturation	95	10 min.	1
Denature	95	10 ~ 30 sec.	30 ~ 45
Anneal	50 ~ 65	10 ~ 30 sec.	
Extend	72	10 ~ 60 sec.	
Final Extension	72	5 min.	1

NOTE: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

5. After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

RUO Research Use Only