

WizPure™ RT-PCR 2X Master

RUO For Research Use Only

REF W2401

DESCRIPTION

WizPure™ RT-PCR 2X Master combines all the reagents necessary for successful routine RT-PCR in a convenient one-step format. The RT-PCR 2X Master is an optimized, ready-to-use PCR mixture of WizScript™ RTase, HS-Taq DNA Polymerase, PCR buffer, MgCl₂ and dNTP's, except RNA template and primers.

The master-mixed formulation saves time and reduces potential contaminating errors by eliminating several pipetting steps. WizPure™ RT-PCR 2X Master is specifically designed to amplify targets up to 1.0 kb and is free of detectable nonspecific nucleases. An enhanced buffer allows for RT reaction temperatures up to 50°C. This can improve detection of more difficult targets as higher RT temperatures reduce nonspecific priming and facilitate melting of RNA secondary structures.

RT-PCR converts and amplifies single-stranded RNA template yielding 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.

KIT CONTENTS

Contents	W2401	W2401-5
WizPure™ RT-PCR 2X Master	1 ml	5 X 1 ml

APPLICATIONS

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

STORAGE CONDITIONS

Upon receipt, store all components at -20°C.

Store the Master mix at 4°C after thawing for up to 6 months, depending on the expiration date, without showing any reduction in performance.

NOTE

Do not contaminate the WizPure™ RT-PCR 2X Master with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

QUALITY CONTROL ANALYSIS:

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizPure™ RT-PCR 2X Master kit is tested against predetermined specifications to ensure consistent product quality.

PROTOCOL

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the RT-PCR 2X Master mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
3. The following table shows recommended component volumes:

Component	20 µl reaction	Final Conc.
RT-PCR 2X Master	10 µl	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	

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

NOTE: Recommended amount of template per PCR reaction:

- < 50 ng plasmid or
- < 500-1000ng genomic DNA or
- 2µl of a 100µl single plaque eluate or
- one single bacterial colony

4. 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 heated lid on thermal cycler.

5. Transfer tubes into a PCR instrument and run as following table.

Step	Temp (°C)	Time	Cycle
cDNA synthesis	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.

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

ORDERING INFORMATION

Product	Cat No.	Package
WizPure™ RT-PCR 2X Master	W2401	100 rxn
	W2401-5	500 rxn

Technical Support



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