www.real-biotech.com
Real Biotech Corporation
13F.-2, No.33, Sec. 1, Minsheng Rd., Banqiao City, Taipei County 220, Taiwan, R. O. C.
Tel: +886 2 2950 9000 Fax: +886 2 2950 0505

RealSens™ Real-Time PCR Mastermix (For Probe System w/ ROX)

Description

RealSens™ Real-Time PCR Mastermix is supplied as 2-fold concentrated, ready-to-use mixture which is highly sensitive and optimized for use with any real-time PCR cycler and sequence-specific probes (ex: Taqman, Molecular Beacon…etc). It contains all the factors needed to perform real-time PCR, including RBC SuperTaq® HotStart DNA Polymerase, RBC Taqman real-time PCR buffer, ROX passive reference dye, dNTPs and 5mM $MgCl_2$. The only step to perform real-time PCR is to add the primers, probe and template. With unique composition of RBC SuperTaq® HotStart DNA Polymerase and RBC Taqman real-time PCR buffer, RealSens™ Real-Time PCR Mastermix provides highly sensitive detection and accurate quantification. It makes real-time PCR simple and easy.

Specifications

Cat. No.	Product Name	Specification
RT601	RealSens™ Real-Time PCR Mastermix (For Probe System w/ ROX), 100 reactions	2X RealSens™ Real-Time PCR Mastermix:1.25ml
RT602	RealSens™ Real-Time PCR Mastermix (For Probe System w/ ROX), 400 reactions	2X RealSens™ Real-Time PCR Mastermix: 5ml
RT603	RealSens™ Real-Time PCR Mastermix (For Probe System w/ ROX), 800 reactions	2X RealSens™ Real-Time PCR Mastermix: 10ml

Features

- Use of any sequence-specific probe on any real-time cycler
- Highly sensitive detection and accurate quantification
- Optimized, ready-to-use mixture format makes real-time PCR simple and easy

Content

- RBC SuperTaq® HotStart DNA Polymerase
- RBC Taqman real-time PCR buffer
- ROX passive reference dye
- dNTP mix including dATP、dCTP、dGTP、dTTP
- 5mM $MgCl_2$

Quality Control

Tenfold serial dilution (10^9~10^{10}) of plasmid DNA were amplified using primers specific to the NNV gene. Triplicate reactions at each concentration were amplified along with no-template controls. Standard curve is r=0.995, efficiency=94.8% and standard deviation of Ct<1.0.

Applications

RealSens™ Real-Time PCR Mastermix is compatible with all available real-time cyclers, including instruments from Applied Biosystems, Bio-Rad, Roche…etc. It is optimized for quantitative real-time PCR and two-step RT-PCR using Probe detection format.

Shipping and Storage Conditions

RealSens™ Real-Time PCR Mastermix is shipped on dry ice and should be stored immediately upon receipt at -20℃ in a constant temperature freezer and protected from light. Avoid repeated freeze–thaw cycles. With proper storage and handled correctly, RealSens™ Real-Time PCR Mastermix can be stored for up to 12 months without showing any deduction in performance and quality.

Protocol

General Reaction Conditions

Our protocol is for a reaction size of 25ul. This protocol serves only as a guideline for real-time PCR amplification. Optional reaction conditions may vary and must be individual determined.

Notes: Use disposable tips containing hydrophobic filters to minimize cross-contamination.

1. Thaw the reagents completely, vortex well and then add following components to a sterile microtube on ice:

Component	Volume/ Reaction	Final Concentration
2X RealSens™ Real-Time PCR Mastermix	12.5 µl	1X
Forward Primer (10µM)	0.75µl	0.3~0.6µM
Reverse Primer (10µM)	0.75µl	0.3~0.6µM
RNase-Free Water	Add to 23.0µl	

2. Mix above components thoroughly by pipetting up and down and dispense the 23µl of mixture into PCR tubes or plates.
3. Add 2µl of the DNA or cDNA and mix carefully by pipetting up and down.
4. Suggested Real-Time Cycler Conditions. The temperature of X℃ should be above the Tm of Primer dimmers, but around 3℃ below the Tm of the specific PCR product. (Tm dimmer < X < Tm product).

For the target gene shorter than 300 bp:

Segment	Number of Cycles	Temperature	Time
1	1	95℃	10 minutes
2	40~45	95℃	15 seconds
		X℃*	30 seconds
3	1	4℃	∞
* X: optimal annealing temperature is depending on user's primer sequences.			

For the target gene longer than 300 bp:

Segment	Number of Cycles	Temperature	Time
1	1	95℃	10 minutes
2	40~45	95℃	15 seconds
		X℃*	25 seconds
		72℃**	10 seconds
3	1	4℃	∞
* X: optimal annealing temperature is depending on user's primer sequences. ** It takes around 1 minute for amplifying 1kb product at 72℃.			

5. Place the PCR tubes or PCR plates in the thermal cycle and start the cycling program.
6. Perform a melting curve analysis of the PCR products.