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

Description

PairFast™ Real-Time PCR Mastermix is especially designed for amplifying DNA template below 300 bp in a fast PCR mode. It's supplied as 2-fold concentrated, ready-to-use mixture which is highly sensitive and optimized for use with any real-time PCR cycler and any sequence-specific probes (ex: Taqman, Molecular Beacon…etc). It contains all the factors needed to perform real-time PCR, including RBC SuperiorTaq® 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 SuperiorTaq® HotStart DNA Polymerase and RBC Taqman real-time PCR buffer, PairFast™ Real-Time PCR Mastermix provides fast, highly sensitive detection and accurate quantification. It makes real-time PCR quick, simple and easy.

Specifications

Cat. No.	Product Name	Specification
RT801	PairFast™ Real-Time PCR Mastermix (For Probe System w/ ROX), 100 reactions	2X PairFast™ Real-Time PCR Mastermix: 1ml
RT802	PairFast™ Real-Time PCR Mastermix (For Probe System w/ ROX), 400 reactions	2X PairFast™ Real-Time PCR Mastermix: 4ml
RT803	PairFast™ Real-Time PCR Mastermix (For Probe System w/ ROX), 800 reactions	2X PairFast™ Real-Time PCR Mastermix: 8ml

Features

- High PCR specificity with unique composition of RBC SuperiorTaq® HotStart DNA Polymerase
- Fast, highly sensitive detection and accurate quantification for DNA template below 300 bp
- Optimized, ready-to-use mixture format makes real-time PCR quick, simple and easy

Content

- RBC SuperiorTaq® 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=0.999, efficiency=92.4% and standard deviation of Ct<1.0.

Applications

PairFast™ 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

PairFast™ 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, PairFast™ 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 20ul. 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. Prepare the reaction mixture on ice.

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

Component	Volume/ Reaction	Final Concentration
2X PairFast™ Real-Time PCR Mastermix	10μl	1X
Forward Primer (10μM)	0.6~1.2μl	0.3~0.6μM
Reverse Primer (10μM)	0.6~1.2μl	0.3~0.6μM
Probe (10μM)	0.4~0.8μl	0.2~0.4uM
RNase-Free Water	Add to 18.0μl	

3. Mix above components thoroughly by pipetting up and down and dispense the 18μl of mixture into PCR tubes or plates.

4. Add 2μl of the DNA or cDNA and mix carefully by pipetting up and down.

5. Suggested Real-Time Cycler Conditions.

Segment	Number of Cycles	Temperature	Time
1	1	95℃	20 seconds
2	40~45	95℃	3 seconds
		58~65℃*	≧20 seconds
3	1	4℃	∞
*Optimal annealing temperature is depending on user's primer sequences. Suggested annealing temperature is above the Tm of Primer dimmers, but approximately 3℃ below the Tm of the specific PCR product. (Tm dimmer < annealing temp. < Tm product).			

6. Place the PCR tubes or PCR plates in the thermal cycle and start the cycling program.

7. Perform data analysis of the PCR products.