



PairFast™ Real-Time PCR Mastermix (For SYBR Green 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 using SYBR Green detection format. It contains all the factors needed to perform real-time PCR. The only step to perform real-time PCR is to add the primers and template. With unique composition of RBC SuperiorTaq® HotStart DNA Polymerase and RBC SYBR Green 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
RT701	PairFast™ Real-Time PCR Mastermix (For SYBR Green System w/ ROX), 100 reactions	2X PairFast™ Real-Time PCR Mastermix: 1ml
RT702	PairFast™ Real-Time PCR Mastermix (For SYBR Green System w/ ROX), 400 reactions	2X PairFast™ Real-Time PCR Mastermix: 4ml
RT703	PairFast™ Real-Time PCR Mastermix (For SYBR Green 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
- SYBR Green I dye
- ROX passive reference dye
- RBC SYBR Green real-time PCR buffer
- dNTP mix including dATP、dCTP、dGTP、dTTP
- 5mM MgCl₂



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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 SYBR Green 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°C 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
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°C	20 seconds
2	40~45	95°C	3 seconds
		58~65°C *	≥ 20 seconds
3	1	4°C	∞

*Optimal annealing temperature is depending on user's primer sequences. Suggested annealing temperature is above the T_m of Primer dimmers, but approximately 3°C below the T_m of the specific PCR product. (T_m dimer < annealing temp. < T_m product).

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