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HOT-RESCUE REAL TIME PCR KIT-FLUOPROBE MBK0012

400 REACTIONS

PURPOSE OF THE KIT

The Hot-rescue Real Time PCR kit is an optimized solution for real-time PCR assays, using probe chemistry etc.

KIT CONTENTS

Component	volume
2X Hot-Rescue Real Time master mix	4 x 1250 µl
Hot-Rescue PLUS DNA Polymerase (5U/µl)	1 x 250 U
ROX	4 x 8 ul

Hot-Rescue Real Time master mix for real time PCR analysis is supplied in a 2X concentration. The mix contains:

- Optimized buffer components
- $MgCl_2$,
- dNTP's

ADDITIONAL EQUIPMENT

- Disposable latex gloves.
- Precision pipettes.
- Aerosol resistant pipette tips.
- Sterile 1.5 ml tubes.
- PCR grade Water.
- Plastic PCR tube or 96-well reaction plates that are recommended by the manufacturer of the quantitative PCR instrument.
- Thermal cycler for quantitative PCR.

STORAGE

Store the kit at –20°C and protect ROX solution from light.
Repeated thawing and freezing cycles may reduce the sensitivity and should be avoided.
It is suggested to freeze the reagents in aliquots for intermittently use.

PRECAUTIONS

- Utilize DNA template free from PCR inhibitors.
- Reduce pipetting errors using calibrated pipets.
- Gently mix the reaction avoiding bubbles.
- Dispense appropriate volume into PCR tubes or plates.
- Use a cap seating tool or firm finger pressure to properly seat caps or film sealer.
- Avoid direct finger contact of the cap or sealing film and optical surface.
- Since bubbles can interfere with fluorescence detection it may be necessary to centrifuge plates or single tube before PCR run.

GUIDELINES

- Follow the guidelines below to achieve optimal PCR performance:
- Reaction volume: 25-100 µl.

- Template: do not use more than 1 µg genomic DNA in a 100 µl reaction.
- Primer and Probe design: see guidelines recommended by producers.
- Amplicon size: the best results are obtained with 100-400 bp length.
- $MgCl_2$: 1x master mix contains 5 mM $MgCl_2$

PROCEDURE

1. PCR Mix preparation

• Upon first use of 2X Hot-Rescue Real Time master mix, it is necessary to add ROX for instruments that require a passive reference dye, as follows:
-Low Rox: ABI Prism® 7500, QuantStudio 3, Quant studio 5
-High Rox: StepOne, StepOne Plus, ABI Prism® 7700
-No Rox: Rotor-Gene Q

• The 2X Hot-Rescue Real Time master mix should be completed immediately before the use according to the instruction below:

-For Low Rox instruments → add 72 µl of PCR grade Water to the provided vial containing 8 µl ROX and vortex for 30". Proceed by completing the 2X Hot-Rescue Real Time master mix with the addition of 6,25 µL diluted ROX.

-For High Rox instruments → the ROX provided in the kit is ready to-use (no dilution is required). Proceed by completing the 2X Hot-Rescue Real Time master mix with the addition of 6.5 µL ROX not diluted.

Note: the kit provides four separate vials of ROX, one for each vial of 2X Hot-Rescue Real Time master mix. It is recommended to complete the 2X Hot-Rescue Real Time master mix with the correct amount of ROX just before use. The ROX cannot be stored after the preparation.

• Vortex for 15" the completed 2X Hot-Rescue Real Time master mix and centrifuge briefly.

2. Reaction Mix Preparation

Set up the experimental reaction by adding the following components in order:

Component	Volume (µL) for One Reaction	Final concentration
PCR grade Water	Variable	-
2x Hot Rescue Real Time master mix	12,5	1x
Upstream primer	Variable	50−1200 nM*
Downstream primer	Variable	50−1200 nM*
Probe	Variable	50−300 nM**
DNA Template**	Variable	DNA 50pg to 1µg
Hot Rescue DNA Polymerase	0.125 - 0.2µl	0.625−1 Unit/reaction
Total volume	25 ul	

*For different volumes calculate all components proportionally.

3. Thermal Cycling Parameters

Place the plate or tubes into the instrument and run the appropriate PCR program. The optimization of amplification protocols for each primer/template systems is recommended.
An exemple:

Step	Temperature and times	Additional Comments
Initial activation step	95°C 1 min	Hot-Rescue Plus DNA polymerase is active by this heating step
Denaturation	95°C 15 sec	variable
Annealing/extension	60°C 1 min	variable
Cycle number 30-50		Cycle number depends on the amount of DNA template

*Set up one-step annealing/ extension at ≥ 60°C for 1 minute.

DATA ANALYSIS Follow the instrument manufacturer's instruction for probe-based assays analysis.

TROUBLESHOOTING

Observation	Possible Cause	Suggested Solution
No PCR product	Missing components (e.g. primers, template or DNA Polymerase).	Check the assembly of the reaction.
	Missing essential step in the cycler protocol.	Check the thermal profile
	Detection was not activated or activated at the wrong step.	Ensure that fluorescence detection step takes places during the extension step of the PCR thermal program.
	Insufficient starting template.	Increase template amount if possible.
	Primer concentration too low.	Increase primer concentration.
	Primer and probe degraded.	Check for primer degradation.
	Poor quality template.	Prepare Fresh DNA.
Multiple PCR products	Primer concentration too high	Reduce primer
	Primer and probe degraded	Check for primer
No linear correlation between Ct and log of amount templates	Template amount too high.	Reduce the template amount.
	Template amount too low.	Increase template amount.