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HOT-RESCUE REAL TIME PCR KIT-SG
MBK0011

400 REACTIONS

PURPOSE OF THE KIT

Hot-Rescue Real Time PCR Kit is formulated to provide sensitive, specific and easy to use system for quantitative real time analysis of nucleic acid from various source. The double-stranded DNA binding dye SYBR® Green I provide a generic and time-saving method for PCR product detection, since it requires only a set of specific primer.

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
25mM $MgCl_2$ Solution	1 x 1000 µl
ROX	4 x 8 ul

Hot-Rescue Real Time master mix for real time PCR analysis is supplied in a 2X concentration and is optimized for SYBR® Green reagent reactions. The mix contains:

- optimized reaction buffer,
- dNTPs,
- $MgCl_2$,
- SYBR Green dye.

Hot-Rescue PLUS DNA Polymerase allow flexibility in the reaction setup, including pre-mixing of PCR reagents at room temperature.
The provided MgCl2 solution allows for an easy optimization of the PCR performance. Magnesium concentration indeed affects primer annealing, product specificity and enzyme activity. Generally, a $MgCl_2$ final concentration of 1.5 mM is sufficient for most reactions, but an optimal Mg^{++} concentration (reaching to 5-6 mM) may increase the performance of PCR reaction with cDNA, as well as genomic and plasmid DNA templates.
Careful optimization of the PCR condition contribute to the reduction of primer-dimer formation.

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 recommended by the manufacturer of the quantitative PCR instrument.
- Thermal cycler for quantitative PCR.

STORAGE

Store the kit at –20°C and protect 2x Hot-Rescue Real Time master mix and ROX solution from light.
Repeated thawing and freezing cycles may reduce the sensitivity and should be avoided.

PRECAUTIONS

- Utilize DNA template free from PCR inhibitors.
- Minimize the exposure of the master mix to light.
- 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.
- To amplify more than 10 ng of genomic DNA adjust the reaction volume as indicated:
  - 10 - 200 ng of genomic DNA in 50 µl/reaction.
  - 200-1000 ng of genomic DNA in 100 µl/reaction.
- Primer design: use primers with matched Tm. Avoid inter- and intra-primer complementary sequences.
- Amplicon size: the best results are obtained with 100-400 bp length.
- $MgCl_2$: 1x master mix contains 1,5 mM $MgCl_2$

PROCEDURE

1. PCR Mix preparation

• Thaw all required reagents completely, mix well by vortexing and spin prior to use.
• Upon first use of 2X Hot-Rescue Real Time master mix, it is necessary to add ROX <u>for instruments that require a passive reference dye, as follows</u>:
-Low Rox: ABI Prism® 7500, QuantStudio 3, Quant studio 5
-High Rox: StepOne, StepOne Plus
-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:

-<u>For Low Rox instruments</u> → 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.

-<u>For High Rox instruments</u> → 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 5 µL ROX.

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 according to the volumes suggested in the following table
For optimal performance, adjust your primer concentration, MgCl2 and DNA concentration.

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Component	Volume (µL) for 1 Reaction	Final concentration
PCR grade Water	Variable	-
2x Hot Rescue Real Time master mix	12,5	1x
25 mM $MgCl_2$ Solution	Variable	Up to **6** mM
Upstream primer	Variable	**0.1-0.4** µM
Downstream primer	Variable	**0.1-0.4** µM
DNA template	Variable	**0.01-10** ng of genomic DNA
Hot-Rescue Plus DNA Polymerase	0,125ul	**0,625** Units/reaction
Total volume	25 ul	

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 10 min	Hot-Rescue Plus DNA polymerase is active by this heating step
Denaturation	95°C 10-20 sec	The time is variable
Annealing	X°C 10-30 sec	Approximately 5 to 8°C below T_m of primers
Extension	68-72°C 15-45 sec	Variable
Cycle number 30-50		Cycle number depends on the ampunt of template DNA

NOTE:

- Since SYBR Green I fluorescent dye binds to all double-stranded DNA, it is highly recommended to perform routinely a melting curve analysis in order to verify the specificity and the identity of the PCR products.
- Select ROX as passive reference dye for instruments that require it (es. Applied Biosystems).
- Fluorescence is detected during annealing-extension step on green channel (FAM dye)

DATA ANALYSIS

Follow the instrument manufacturer's instruction for SYBR Green I dye 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 thermal profile.	Check the thermal profile.
	Detection was not activated or activated in the wrong step.	Ensure that fluorescence acquisition step takes place during the extension step of the PCR thermal program.
	Annealing temperature too high.	Decrease annealing temperature
	Insufficient starting template.	Increase template amount if possible
	Primer concentration too low.	Increase primer concentration.
	Insufficient extension time for the amplicon size.	Increase the extension time.
	Poor quality template.	Prepare Fresh DNA.
Multiple PCR products	Annealing temperature too low.	Increase annealing temperature.
	Primers concentration too high	Reduce primer concentration.
	Primers not optimal	Check the primers design.
	Mg^{++} concentration not optimal.	Vary Mg^{++} concentration up to 6 mM.
	Extension time too long.	Decrease extension time.
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.
	Primer-dimers co-amplified with target	Decrease the annealing temperature, primer concentration or MgCl2 concentration.