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002059 Rev A

Primer & Probe Concentration (Multiplexing) *(Cont'd)*:

- Starting concentrations of 400 nM are suitable for most assays.
- Primer concentration should be varied to achieve optimum qPCR assay efficiencies (90 – 110%) for the target.
- Probe concentrations: 250 nM is suitable for most targets. For high abundance targets: optimize the reaction using probe concentrations ranging from 100 – 250 nM.

Template Preparation & Concentration:

- Compatible templates: cDNA, gDNA, and plasmid. No purification is required for use of cDNA with the Sahara Multiplex qPCR Master Mix.
- Mix is highly resistant to PCR inhibition—use with purified DNA provides maximum resistance.
- A range of 50 ng – 2 pg is typical for large genomes (i.e. human, mouse). The range of input DNA for smaller genomes (i.e. yeast, viruses) is greater. Master mix dynamic range: 10^{10} copies – 1 copy. Adjust input DNA as necessary.

Carryover Contamination Prevention:

- False positives may result from minimal amounts of contaminating DNA. The Sahara Multiplex qPCR Master Mix contains dUTP. Treating reactions with UNG (provided by user) during setup will prevent false positives by selectively degrading dUTP-containing templates.

TROUBLESHOOTING

Little/no product detected:

- Reagent was omitted from or improperly added to the assay. Verify complete adherence to protocol steps. Further optimization of PCR protocol may be required.
- Improper qPCR channel selection. Ensure channel assignment matches dye/probe.

Inconsistent replicate amplifications:

- Pipetting error. Ensure proper technique.
- Insufficient mixing during preparation. Ensure thorough mixing of original reagents and qPCR reaction contents.

Inconsistent plasmid amplifications:

- Supercoiling of targets. Linearize the plasmid.

Efficiency out of range (< 90% or > 110%) OR Unexpected R^2 value:

- Pipetting or dilution error during assay setup.
- qPCR interference due to bubbles.
- Suboptimal reaction conditions.
- Inappropriate instrument threshold setting.

Amplification of No Template Control (NTC):

- Reagent contamination by DNA template.

Excessive primer dimer formation:

- Suboptimal primer concentration or design. Redesign assay using software to check for primer dimer formation across all assays.

CHAI

Sahara Multiplex qPCR Master Mix 2X

Catalog #: R02210

For research use only

Store at:
-20 °C long-term
4 °C for 6 months

Avoid repeated freeze/thaw cycles
Protect from light

INTRODUCTION

Sahara Multiplex qPCR Master Mix is a 2X universal mix for quantitative probe-based detection using Real-Time PCR. The versatility of the Sahara Multiplex Master Mix eliminates the need for countless specialized mixes. Aptamer-based hot start technology, unparalleled thermal stability, compatibility with targets from an expansive range of organisms and inhibition resistance maximized for use with crude extracts and environmental samples make the Sahara Multiplex qPCR Master Mix a comprehensive qPCR master mix solution.

HIGHLIGHTS

- Offers high resistance to PCR inhibition even with crude extracts & environmental samples
- Retains stability even under extreme conditions: three months at 25 °C, eight days at 50 °C, and 20 freeze/thaw cycles
- Multiplex up to four reactions without any effect on C_q
- Amplifies GC-rich targets with up to 70% GC-content
- Supports fast protocols with demonstrated minimum cycling times as low as 20 minutes for 30 cycle quantitative protocols or 40 cycle presence/absence protocols

SPECIFICATIONS

Polymerase	Taq DNA Polymerase
GC-Rich Performance	≤ 70%
ROX Reference Dye	Optional—Included Separately
Hot Start	Yes—Aptamer
Fast Protocols	Presence/absence: 20 min/40 cycle Quantitative: 20 min/30 cycle
Supported Probes	TaqMan/Hydrolysis, Molecular Beacon, Scorpions
Supported Templates	cDNA, Genomic DNA, Plasmid DNA
Carryover Prevention	Yes—dUTPs Included

PROTOCOL

Prepare Master Mix:

- Thaw completely at room temperature. Mix contents by inversion or pipetting, or by gently vortexing for < 3 s.
- Centrifuge to collect the solution at the bottom of the tube.
- For instruments requiring ROX, add appropriate quantity of ROX reference dye to master mix stock according to quantities indicated. Invert several times to mix.

STOCK VIAL SIZE	HIGH ROX SYSTEM	LOW ROX SYSTEM
1 mL	20 µL	2 µL
5 mL	100 µL	10 µL

Reaction Setup:

COMPONENT	20 µL REACTION	FINAL CONC.
Sahara Multiplex qPCR Master Mix	10 µL	1X
Forward & reverse primers	variable	50 – 1000 nM
Probe(s)	variable	100 – 250 nM
Template DNA	variable	< 100 ng
Nuclease-free Water	to 20 µL	

NOTE: Based on 20 µL reaction, adjust accordingly for other volumes.

- Determine total volume required for reactions + 10%.
- Prepare assay mix for all components except template.
- Aliquot reaction mix into qPCR tubes/plate.
- Add DNA template to qPCR tubes/plate.
- Close tubes/seal plate.
- Spin briefly to remove bubbles and collect liquid at bottom.

Thermocycling Conditions:

STEP	TEMP.	TIME
Initial Denaturation	95 °C	1 min
Denature	95 °C	15 s
Extend	60 °C	30 s

● Cycle 30 - 45x

USAGE NOTES

Assay Design:

- Amplicon length for standard protocols: amplicon lengths of 80 – 200 bp produce optimal reaction efficiency values.
- GC content: Sahara Multiplex qPCR Master Mix is compatible with GC content from 40 – 70%. Proprietary mix components enhance performance of assays using GC-rich templates.
- Annealing temperature: a 60 °C annealing and extension temperature is recommended for two-step Real-Time PCR. If the T_m of the primer is < 60 °C: perform a three-step PCR with annealing temperature set lower than the T_m of the primer. Anneal for 10 – 20 s and extend at 72 °C.
- Extension time: extension rate = 1 kb/min.

Fast Protocols:

- Amplicon length should be 50 – 100 bp.
- Reduce initial denaturation to 30 s, denaturation step to < 15 s, and extension step to 15 – 20 s.

Primer & Probe Concentration (Multiplexing):

- Optimize primer concentration for each individual target as a singleplex assay. Execute multiplex assay with all targets. Ensure there is no change in C_q. Ensure all targets are amplified: a target present at a higher concentration relative to another target may require a relatively lower corresponding primer concentration.