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002111 Rev A

USAGE NOTES

Assay Design – Endpoint PCR:

- Amplicon length: amplicons up to 4000 bp may be amplified.
- Primer concentration: 100 nM is suitable for most assays. May be varied between 50 – 1000 nM.

Template Preparation:

- Compatible templates: cDNA, gDNA, and plasmid. No purification is required for use of cDNA with the Sahara Hot Start PCR Master Mix.
- Purified DNA is ideal as PCR inhibition is avoidable. Inhibition may occur with crude preparations despite inhibition resistance by the mix, resulting in a higher limit of detection.
- A range of 50 ng – 2 pg is typical for large genomes (i.e. human, mouse). The range of input DNA for smaller genomes is greater.
- GC content: Sahara Hot Start PCR Master Mix is compatible with GC content from 40 – 70%.

Cloning:

- Template concentration: may be increased up to 1000 ng to generate maximum product.
- 3' adenine overhang: a 3' dA overhang is generated by the Taq polymerase, allowing amplified products to be ligated using TA cloning.

TROUBLESHOOTING

Inconsistent plasmid amplifications:

- Supercoiling of targets. Linearize the plasmid.

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.
- Check melt temperature of primers and lower the annealing temperature if needed.
- Improper qPCR channel selection. Ensure channel assignment matches probe.

Inconsistent replicate amplifications:

- Pipetting error. Ensure proper technique.
- Insufficient mixing during preparation. Ensure thorough mixing of original reagents. Ensure PCR reaction contents are adequately mixed following setup.

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

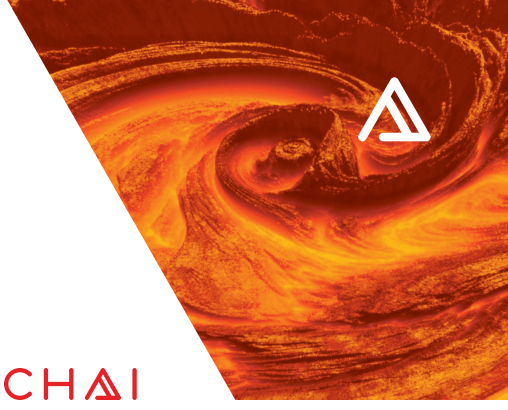
- Pipetting or dilution error during assay setup.
- qPCR interference due to bubbles.
- Suboptimal reaction conditions.
- Inappropriate instrument threshold setting.

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.



Sahara Hot Start PCR Master Mix 2X

Catalog #: R02151

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 Hot Start PCR Master Mix is a 2X mix for endpoint PCR or quantitative probe-based detection using singleplex Real-Time PCR. Aptamer-based hot start technology, unparalleled thermal stability, compatibility with targets from an expansive range of organisms, and high amplification efficiency make it an ideal mix for routine PCR applications.

HIGHLIGHTS

- Retains stability even under extreme conditions: three months at 25 °C or eight days at 50 °C, followed by 20 freeze/thaw cycles
- Aptamer-based hot start system reduces non-specific amplification and primer dimers
- Amplifies up to four kb from genomic DNA, and GC-rich targets with up to 70% GC-content

SPECIFICATIONS

Polymerase	Taq DNA Polymerase
GC-Rich Performance	≤ 70%
Max Amplicon Length	4 kb
Hot Start	Yes—Aptamer
Supported Probes	TaqMan/Hydrolysis, Molecular Beacon, Scorpions
Supported Templates	Genomic DNA, cDNA, Plasmid DNA

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.

Reaction Composition – Endpoint PCR:

COMPONENT	20 µL REACTION	FINAL CONC.
Sahara Hot Start PCR Master Mix	10 µL	1X
Forward & Reverse Primers	variable	50 – 1000 nM
Template DNA	variable	< 1000 ng
Nuclease-free Water	to 20 µL	

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

Reaction Composition – Real-Time PCR:

COMPONENT	20 µL REACTION	FINAL CONC.
Sahara Hot Start PCR Master Mix	10 µL	1X
Forward & Reverse Primers	variable	50 – 1000 nM
Probe	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.

Reaction Setup:

- Determine total volume required for reactions + 10%.
- Prepare assay mix for all components except template.
- Aliquot reaction mix into PCR tubes/plate.
- Add DNA template to PCR tubes/plate.
- Close tubes/seal plate.
- Centrifuge briefly

Thermocycling Protocol – Three-Step:

Recommended for endpoint PCR, or Real-Time PCR with melt temperatures < 60 °C.

STEP	TEMP.	TIME
Initial Denaturation	95 °C	1 min
Denature	95 °C	15 s
Anneal	45 – 68 °C	15 – 30 s
Extend	72 °C	1 kb/min
Final Extension	72 °C	5 min

● Cycle 30 - 45x

Thermocycling Protocol – Two-Step:

Recommended for Real-Time PCR with melt temperatures ≥ 60 °C.

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 – Real-Time PCR:

- Amplicon length: amplicon lengths of 80 – 200 bp produce optimal reaction efficiency values.
- Annealing temperature: a 60 °C annealing and extension temperature is recommended for two-step Real-Time PCR. Perform three-step PCR if the T_m of the primer is < 60 °C.
- Primer concentration: 400 nM is suitable for most assays. Primer concentration should be varied to achieve optimum PCR 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.

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