SolisFAST® Master Mix with UNG, Ready To Load, 5x

Catalogue Number	Pack Size	20 µl rxn
24-22-0000S	0.2 ml	50
24-22-00001	1 ml	250
24-22-00001-5	5 x 1 ml	5 x 250
24-22-00020	20 ml	5000

Shipping:
At room temperature

Store at -20 °C

Stability at room temperature 1 MONTH

Batch Number and Expiry Date:
See vial

Storage and Stability:
- Routine storage at -20 °C until expiry date
- The mix can be stored at +4 °C for up to 6 months
- Stability at room temperature (15 - 25 °C) for 1 month
- Freeze-thaw stability: 30 cycles

Reaction setup:
At room temperature

Manufactured by Solis BioDyne, in compliance with the ISO 9001 and ISO 13485 certified Quality Management System.

Product description:

- SolisFAST® Master Mix with UNG, Ready To Load, is a 5x-concentrated, ready-to-use solution for fast and sensitive singleplex and multiplex PCR assays.
- Allows amplification of up to 5 kb fragments from low complexity DNA templates (e.g. cDNA, lambda, plasmid DNA), and up to 3 kb from genomic DNA (human, animal, plant).
- Contains all components necessary, except primers, nuclease-free water, and DNA template.
- Includes a loading dye enabling PCR products to be loaded directly on a gel. Contains dUTP instead of dTTP, as well as uracil-N-glycosylase (UNG) to prevent carry-over contamination from previous amplifications.

Mix component	Description
SolisFAST® DNA Polymerase	*In silico* designed analogue of *Taq* DNA polymerase with enhanced stability at room temperature, fast hot-start and faster extension rates compared to the wild-type *Taq* DNA polymerase
PCR buffer	Includes 17.5 mM $MgCl_2$ (1x PCR solution 3.5 mM $MgCl_2$), dNTPs (dATP, dCTP, dGTP, dUTP), UNG, additives that maximize efficiency of PCR
Green loading dye[1]	Mix of blue and yellow dye to allow tracking of DNA during electrophoresis

[1] The mix is not recommended for use in applications where spectrophotometric measurements are necessary (e.g. Sanger sequencing). Migration speed: blue dye – TBE: ~3.5 kb, TAE: ~4.5 kb; yellow dye – TBE: ~35 bp, TAE: ~45 bp).

Step-by-step guidelines:

1. Thaw SolisFAST® Master Mix, template DNA, primers, and nuclease-free water. Mix each component by gentle vortexing or pipetting up and down, then centrifuge briefly.
2. Prepare a reaction mix. Add all required components except the template DNA.

   NB! To avoid carry-over contamination, mix all reaction components at room temperature to ensure full activation of the UNG enzyme. Any dUTP-containing amplicon from a previous reaction will then be digested by UNG.

Component	Volume[1]	Final conc.
SolisFAST® Master Mix (5x)	4 µl	1x
Forward Primer(s) (10 µM)	0.5 µl	250 nM
Reverse Primer(s) (10 µM)	0.5 µl	250 nM
Template DNA (added at step 4)	Variable	Variable[2]
Nuclease-free water	up to 20 µl	
Total reaction volume	**20 µl**	

[1] Scale all components proportionally according to sample number and reaction volumes. Make sure you use enough of each reagent for your reactions, plus 10% extra volume to accommodate pipetting errors. For high reaction efficiency, do not exceed 20 µl reaction volumes.

[2] For low complexity templates (i.e. cDNA, plasmid, lambda), use 10 pg – 50 ng of DNA per 20 µl reaction. For higher complexity templates (i.e. gDNA), use 200 pg – 100 ng of DNA per 20 µl reaction.

3. Mix the reaction mix thoroughly, then centrifuge briefly. Dispense appropriate volumes of mix into PCR wells.

4. Add template DNA to the PCR wells. Seal the wells using the procedure recommended for the cycling instrument being used, and centrifuge the reactions briefly.
5. Incubate your PCR reactions in thermal cycler as follows:

Use **2-step protocol** for singleplex PCR and/or with targets up to 500 bp.
Use **3-step protocol** for multiplex PCR and/or with targets longer than 500 bp.

Step		2-step protocol		3-step protocol	
		Temperature	Time	Temperature	Time
Initial denaturation[1]		98 °C	2 min	98 °C	2 min
30 cycles	Denaturation	98 °C	5 sec	98 °C	10 sec
	Annealing[2]	55 – 65 °C	20 sec	55 – 65 °C	10 sec
	Extension[2]			72 °C	30 sec
Final extension				72 °C	1 min

[1] If you set up reactions on ice, use additional UNG incubation at 25 °C for 5 minutes prior to initial denaturation. Initial incubation at 98 °C is needed for the activation of polymerase, inactivation of heat-labile UNG enzyme, and denaturation of template DNA. With low complexity templates (i.e. cDNA, lambda, plasmid DNA), initial denaturation time can be reduced (30 sec-1 min). Complex templates, such as gDNA, require longer time to denature (2-3 min).

[2] The annealing (in 3-step protocol) and annealing/extension (in 2-step protocol) temperature depends on the melting temperature of the primers. For targets longer than 2 kb, extension should be increased to 1 min or 15-30 sec/kb.

IMPORTANT: To avoid degradation of PCR products by residual activity of UNG enzyme, amplification products should be stored at –20 °C. Storage at room temperature for 24 hours has no detrimental effect on PCR products.

Recommendations for a successful PCR experiment

Prerequisites for a successful PCR experiment include the design of optimal primer pairs, the use of high-quality template DNA and appropriate concentrations of reaction components. Below are a few recommendations that may help to achieve the best results.

Primers

Use dedicated software, such as Primer3 (bioinfo.ut.ee/primer3) or NCBI Primer-BLAST (ncbi.nlm.nih.gov/tools/primer-blast) to design target-specific primers.

The optimal primer length is 20 – 30 bp, with GC-content 35 – 65% and calculated melting temperatures (Tm) 60 – 70 °C. Tm of the two primers should not differ by more than 3 °C.

Analyze your primers for self-complementarity and stable secondary structures. Avoid the 3'-self complementarity, because it increases probability of primer-dimers formation.

In case of multiplexing, check the primers for cross-reactivity and minimize Tm mismatches. Test the efficiency of your PCR experiment in individual assays before combining them in a multiplex assay.

Template

The integrity, purity and concentration of the DNA template should be suitable for the PCR experiment.

The recommended final concentration of DNA template for the qPCR experiment is dependent upon the type of DNA used. Use up to 100 ng of complex (e.g. eukaryotic) DNA and up to 50 ng of cDNA in your reactions.

To monitor possible contamination and primer-dimer formation, always include a no-template control (NTC) by replacing the DNA template with the same volume of nuclease-free water.

Please see the Troubleshooting Guide below for suggestions and help with specific problems.

Troubleshooting Guide

No or low PCR yield

- SolisFAST® DNA Polymerase was not activated and/or template DNA was not fully denatured – make sure that your PCR starts with an initial incubation at 98 °C.
- Cycling conditions are not optimal – decrease the primer annealing temperature; if needed determine the optimal annealing temperature by running a temperature gradient; increase the extension time (if amplifying a long target); increase the number of cycles by 3-5.
- Poor quality of template – check the template's purity and integrity, ensure that your template doesn't contain PCR inhibitors.
- Template concentration is too low – increase the concentration of DNA template.
- Primer concentration is not optimal – titrate primer concentration (final concentration 150-350 nM of each); ensure that both primers have the same concentration.
- Primers are degraded – check the quality of the primers
- SolisFAST® Master Mix is degraded – check the storage conditions and expiry date of the Mix; perform a positive control with template DNA and primers previously known to amplify.

Non-specific products

- Non-specific amplification – ensure that your primers are target-specific.
- Primer concentration is not optimal – titrate primer concentration (final concentration 150-350 nM of each); too high primer concentration can reduce the binding specificity, resulting in unwanted products.
- Primer annealing temperature is too low – increase the annealing temperature; keep your primer annealing temperature 2-5 °C below the Tm of the primer having the lowest Tm.
- Too many cycles – reduce the cycle number by 3-5 to remove non-specific bands.
- Contamination – to avoid contamination, work in dedicated space, keep pre- and post-amplification areas separate, use personal protective equipment, decontaminate your surfaces and equipment, if possible, aliquot your reagents into smaller volumes to prevent contamination of stock solutions.

Smearing in electrophoresis

- Too much template – load lower amount or prepare serial dilutions of template.
- Too many cycles – reduce the cycle number by 3-5.
- Extension time is too long – reduce extension time.
- Primer design is not optimal – review your primers and redesign the primers if needed.

Safety precautions:

Please refer to Safety Data Sheet for more information.

Technical support:

Contact your sales representative for any questions or send an email to **support@solisbiodyne.com**

Revised 11.11.2019

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