SolisFAST® Probe qPCR Mix with UNG (no ROX), 5x

Catalogue Number	Pack Size	20 µl rxn
28-21-0000S	0.2 ml	50
28-21-00001	1 ml	250
28-21-00001-5	5 x 1 ml	5 x 250
28-21-00020	20 ml	5000

Shipping:
At room temperature

Store at –20 °C upon receipt

Batch Number and Expiry Date:
See vial

Storage and Stability*:
- Routine storage at –20°C (–28°C to –18°C) until Expiry Date
- Stable at 4°C (2°C to 8°C) for 12 months
- Stable at room temperature (25°C) for 3 months
- 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® Probe qPCR Mix with UNG (no ROX) is a 5x-concentrated, ready-to-use solution for fast, highly sensitive and reproducible probe-based qPCR assays using dual-labeled hydrolysis probes (e.g. TaqMan® probes), and is suitable for detection and quantitation of up to five targets simultaneously.
- The mix contains all components necessary, except primers, probe(s), nuclease-free water, and DNA template.
- Contains dUTPs instead of dTTPs, as well as Salini UNG™ 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 due to a genetic modification – Stability TAG**, fast hot-start and faster extension rates compared to the wild-type *Taq DNA* polymerase
Salini UNG™ Uracil-N-Glycosylase (UNG)	Heat-labile UNG that eliminates uracil from dUTP-DNA by catalyzing the hydrolysis of the N-glycosylic bond and creating an abasic site
qPCR buffer	Includes 16.5 mM $MgCl_2$ (1x PCR solution 3.3 mM $MgCl_2$), dNTPs (dATP, dCTP, dGTP, dUTP), UNG, additives that maximize efficiency of PCR

Compatible real-time instruments:

The mix is compatible with qPCR cyclers that do not require an internal reference dye (e.g., ROX) for normalization of fluorescent signal (see the compatibility table on page 7).

Step-by-step guidelines:

1. Thaw SolisFAST® Probe qPCR Mix with UNG, template DNA, primers, probe(s), 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® Probe qPCR Mix with UNG (no ROX) (5x)	4 µl	1x
Forward Primer(s) (10 µM)[2]	0.8 µl	400 nM
Reverse Primer(s) (10 µM)[2]	0.8 µl	400 nM
Probe(s) (10 µM)[2]	0.3 µl	150 nM
Template DNA (added at step 4)	Variable	cDNA: < 100 ng gDNA: < 50 ng
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.

[2] Optimal results may require titration of primer concentration between 200 and 600 nM, and probe concentration between 100 and 200 nM. A final concentration of 400 nM each primer and 150 nM probe is suitable for most applications.

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.
5. Seal the wells using the procedure recommended for the cycling instrument being used, and centrifuge the reactions briefly.
6. Program the thermal cycler using the cycling conditions recommended below. Confirm the appropriate detection channel(s) selected for the fluorophore(s) used in the assay.

Step	Temperature	Time	Cycles
Initial denaturation[1]	95°C	3 min	1
Denaturation[2]	95°C	5 sec	40
Annealing/extension[2]	60–65°C	20 sec	

[1] If you set up reactions on ice, use additional UNG incubation at 25°C for 5 minutes prior to initial denaturation. Incubation at 95°C is crucial for a full activation of SolisFAST® DNA Polymerase and denaturation of template DNA. With low-complexity templates (e.g., cDNA) shorter denaturation time (30 sec–1 min) can be used. Complex templates, such as gDNA, require longer time to denature (2–3 min).

[2] The annealing/extension temperature depends on the melting temperature of the primers and DNA probe used. 5 sec denaturation and 20 sec annealing/extension are suitable for all qPCR cyclers listed on page 7. The program can be optimized depending on the instrument. Annealing/extension time can be reduced to 10 sec for Bio-Rad CFX96, 5 sec for Mic qPCR cycler (Bio Molecular Systems) (see example on page 7).

7. Place the reactions into the qPCR cycler, and start the qPCR run.
8. After the reaction is completed, perform data analysis according to the instrument-specific instructions.

Recommendations for a successful qPCR experiment

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 and probes. Analyze your primers and probes for self-complementarity and stable secondary structures (e.g., hairpins) in their sequences. Avoid the 3'-self complementarity, because it increases the probability of primer-dimers formation. GC-content of primers and probe(s) should range from 35–65%.

In case of multiplexing, test the performance of primer–probe sets in individual assays before combining them in a multiplex assay.

Check the qPCR cycler's user manual for correct setup for multiplex analysis.

Primers

1. The optimal primer length is 20-30 bp targeting amplicons of 50–150 base pairs. Avoid runs of more than four consecutive G or C bases.
2. The optimal melting temperature (Tm) of the primers is 60–64°C. In order for both primers to bind efficiently, Tm of the two primers should not differ by more than 3°C.

Probe

1. The length of the probes should be 9–40 bases. For the hydrolysis probes, it is usually up to 30 bases. The probe binding site should be in close proximity to the forward or reverse primer. However, the probe and primer binding sites should not overlap. Probe can bind to either strand of the target.

2. Melting temperature (Tm) of the probe should be 6–8°C higher than the Tm of the primers. In case the Tm of the probe is too low, less of the probe is bound to the target sequence. As a consequence, the product may be amplified, but the sensitivity will be compromised, because not all target sites are occupied by the probe, which results in reduced fluorescence signal and not a true representation of the target amount in the sample.

Template

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

1. The template needs to be purified of PCR inhibitors (e.g., EDTA).
2. The recommended final concentration of DNA template for the qPCR experiment is dependent upon the type of DNA used. For example, if you use cDNA as a template, qPCR efficiency would be largely dependent on the expression level of the target gene. Use up to 50 ng of complex (e.g., eukaryotic) DNA and up to 100 ng of cDNA in your reactions.
3. The final amount of cDNA sample in the qPCR reaction is up to one tenth of the final reaction volume. Overload of cDNA sample may inhibit your qPCR reaction.
4. Perform and analyze your qPCR reactions in triplicates on a serially diluted template (e.g., 10-fold dilution series). Dilutions should be done in deionized water and should be prepared fresh before each experiment. Use the standard curve derived from the serial dilutions to assess qPCR efficiency and to determine the optimal template concentration for your qPCR assay.

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

Cycling

The cycling conditions are compatible with qPCR cyclers listed in the following compatibility table:

Manufacturer	Model
Bio-Rad	CFX96™, CFX384™, Opticon™, Opticon2™, MiniOpticon™, Chromo4™, iQ™5, myiQ™
Qiagen	Rotor-Gene™ 3000, Rotor-Gene™ 6000, Rotor-Gene™ Q
Eppendorf	Mastercycler® ep realplex
Bio Molecular Systems	Mic
Takara	Thermal Cycler Dice™ (TP800)
Roche	LightCycler® 96, 480, Nano

Cycling conditions optimized for CFX96™ qPCR cycler (Bio-Rad):

Step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1
Denaturation	95°C	2 sec	40
Annealing/extension	60–65°C	10 sec	

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

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Manufacturer: Solis BioDyne OÜ | Teaduspargi 9, 50411 | Tartu, Estonia (EU)

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