SolisFAST® Probe qPCR Mix with UNG (Purple), 5x

Catalogue Number	Pack Size	20 µl rxn
28-23-0000S	0.2 ml	50
28-23-00001	1 ml	250
28-23-00001-5	5 x 1 ml	5 x 250
28-23-00020	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® Probe qPCR Mix with UNG (Purple) 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 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
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
Passive reference dye	Purple dye is an internal passive reference dye used to normalize the fluorescent reporter signal generated in qPCR

Compatible real-time instruments:
The mix is compatible with qPCR cyclers where Purple dye is used as a passive reference signal for normalization of fluorescent signal (see the compatibility table on page 7).

Step-by-step guidelines:
1. Thaw SolisFAST® Probe qPCR Mix, 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 (Purple) (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. 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 initial 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.

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.