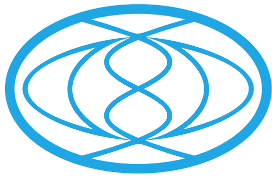

SolisFAST® Lyo-Ready qPCR Kit with UNG

Catalogue Number	Size (20 µl reactions)
28-52-0000S	100 reactions (sample)
28-52-00250	250 reactions
28-52-00250-5	5 x 250 reactions
28-52-05000	5 000 reactions

Store at –20°C upon receipt

Shipping:

On blue ice

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 5 months
- Stable at room temperature (25°C) for 2 weeks
- Freeze-thaw stability: 30 cycles

Reaction setup:

At room temperature

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

Product description:

- **SolisFAST® Lyo-Ready qPCR Kit with UNG** is an optimized qPCR solution compatible with lyophilization. The kit enables fast and sensitive DNA amplification with probe-based qPCR assays. The kit includes a glycerol-free qPCR mix and a lyophilization excipient mix. Blend of the two mixes is suitable for reliable lyophilization into lyo-cakes and lyo-beads. The critical temperature (Tc) of the kit is -30.8 °C and the glass transition temperature (Tg) of the lyophilized product is 68.3 °C.
- **5x SolisFAST® Lyo-compatible qPCR Mix with UNG** is a 5x concentrated glycerol-free qPCR master mix suitable for real-time detection and quantitation of up to five DNA targets simultaneously. The mix includes the SolisFAST® DNA polymerase: an *in silico* designed inhibitor tolerant analogue of *Taq* DNA polymerase with oligo-based hot-start and ~2-4 times faster extension rates compared to the wild-type *Taq*. The mix contains dUTPs instead of dTTPs, as well as Salini UNG® Uracil-N-Glycosylase to prevent carry-over contamination from previous amplifications. The $MgCl_2$ concentration is 16.5 mM (1x mix – 3.3 mM). The mix does not contain any lyophilization excipients.
- **4x SolisFAST® Lyo Excipient Mix** is a 4x concentrated blend of lyophilization additives that protect reagents during freeze-drying and stabilize the lyophilizates. The mix enables lyophilization into cakes and beads.

Kit component	Product sizes and Volumes			
	100 rxn	250 rxn	5x250 rxn	5000 rxn
5x SolisFAST® Lyo-compatible qPCR Mix with UNG	0.4 ml	1 ml	5x 1ml	20 ml
4x SolisFAST® Lyo Excipient Mix	0.5 ml	1.25 ml	5x 1.25 ml	25 ml

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.

Step-by-step guidelines for wet reagent testing:

1. Prepare the DNA sample.
2. Thaw SolisFAST® Lyo-compatible qPCR Mix with UNG, SolisFAST® Lyo Excipient Mix, primers, probe(s), and nuclease-free water.
3. Vortex each component, then centrifuge briefly.
4. Prepare a reaction mix by adding all required components in the exact order as shown in the table below.

Step	Component	Volume[1]	Final conc.
1	Nuclease-free water	Variable	
2	SolisFAST® Lyo-compatible qPCR Mix (5x)	4 µl	1x
3	Forward Primer(s) (10 µM)[2]	0.8 µl	400 nM
4	Reverse Primer(s) (10 µM)[2]	0.8 µl	400 nM
5	Probe(s) (10 µM)[2]	0.3 µl	150 nM
6	Template DNA	Variable	Variable
7	SolisFAST® Lyophilisation Excipient Mix (4x)	5 µl	1x
	Total reaction volume	**20 µl**	

[1] Scale all components proportionally according to number of reactions and total 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 are suitable for most applications.

5. Run the qPCR reactions immediately after reaction setup. Follow the cycling instructions on page 6.

Step-by-step guidelines for lyophilization:

1. Thaw SolisFAST® Lyo-compatible qPCR Mix with UNG, SolisFAST® Lyo Excipient Mix, primers, probe(s), and nuclease-free water. Mix each component by gentle vortexing, then centrifuge briefly.

2. Prepare a reaction mix by adding all required components in the exact order as shown in the table below. The table provides an example protocol for lyophilizing 20 µl reaction volume with primers and probes. If lyophilizing without primers and probes, replace the respective volume with water.

Step	Component	Volume[1]	Final conc.
1	Nuclease-free water	9.1 µl	
2	SolisFAST® Lyo-compatible qPCR Mix (5x)	4 µl	1x
3	Forward Primer(s) (10 µM)[2]	0.8 µl	400 nM
4	Reverse Primer(s) (10 µM)[2]	0.8 µl	400 nM
5	Probe(s) (10 µM)[2]	0.3 µl	150 nM
6	SolisFAST® Lyophilisation Excipient Mix (4x)	5 µl	1x
	Total reaction volume	**20 µl**	

[1] Scale all components proportionally according to number of reactions and total reaction volumes. Make sure you use enough of each reagent for the number of 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. Vortex and centrifuge the reaction mix briefly. Dispense appropriate volumes of the reaction mix to lyophilization vials or other suitable containers (e.g., PCR tubes, plates).

4. Proceed with lyophilization immediately.

5. The following program is suitable for the lyophilization of a 20 µl reaction mix at a 1x concentration. The critical temperature (Tc) of the reagents is set at -30.8 °C. The following parameters are for guidance only and the most optimal lyophilization conditions should be optimized by the user according to the used formats, volumes, and systems. Check page 7 for more lyophilization advice.

Recommended lyophilization protocol for 20 µl reaction volume				
Step	**Shelf temp°**	**Time**	**Pressure, µbar**	**Description**
FREEZING STAGE				
1	+5 °C	30 min	Atmospheric	Hold
2	-45 °C	1 h 40 min	Atmospheric	Ramp (0.5 °C/min)
3	-45 °C	3 h	Atmospheric	Hold
PRIMARY DRYING STAGE				
4	-45 °C	60 min	80 µbar	Hold
5	-40 °C	10 min	80 µbar	Ramp (0.5 °C/min)
6	-40 °C	≥24 h	80 µbar	Hold
SECONDARY DRYING STAGE				
7	+25 °C	2 h 10 min	80 µbar	Ramp (0.5 °C/min)
8	+25 °C	6 h	80 µbar	Hold
STOPPERING STAGE				
9	+25 °C	n/a	500 mbar	Backfill with N_2
10	+25 °C	n/a	500 mbar	Stopper
11	+25 °C	n/a	Atmospheric	Aerate with N_2

6. Prepare the DNA samples in separate tubes in excess. Reconstitute the lyophilized cake or bead by adding **20 µl** of the DNA sample (Check page 7, “Reconstitution”).

7. Vortex until cake or bead is solubilized, then spin down.

8. Perform qPCR according to instructions on page 6.

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

[1] UNG is active at room temperature. If UNG functionality is desired and you are setting up reactions on ice, incorporate an additional UNG step at 25 °C for 1 min before the initial denaturation step.

[2] With low-complexity templates (e.g., cDNA), shorter initial denaturation time (30 sec – 1 min) can be used. Complex templates, such as gDNA, may require longer time to denature (2-3 min).

[3] The cycling program can be optimized depending on the instrument specification, assay design and the desired total run time. Denaturation time between 1 to 5 sec and annealing/extension time between 5 to 20 sec is recommended. Annealing/extension temperature is dependent on the melting temperature of the primers and DNA probe used. Performing a gradient PCR to determine the most optimal annealing/extension temperature is recommended.

Recommendations for a successful qPCR experiment

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. For both primers to bind efficiently, the Tm of the two primers should not differ by more than 3°C. For Tm calculations, use NCBI Blast or Primer3 software.

Probe

1. The length of the probes should be 9–40, optimally ~30 bases. The probe binding site should be near the forward or reverse primer and 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. Otherwise, less of the probe may be bound to the target sequence and fluorescence signal intensities may be decreased.

Template

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

1. For optimal results, it is advisable to use purified DNA. In cases where crude extraction methods are preferred, it is recommended to employ lysis buffers and/or heat treatment and to optimize the sample volume per reaction.

2. The recommended final concentration of DNA template for the qPCR experiment is dependent on the type of DNA used. For example, if using 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 should be up to one tenth of the final reaction volume. Overloading the 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). Use the standard curve derived from the serial dilutions to assess qPCR efficiency and to determine the optimal template concentration for your qPCR assay.

Lyophilization

1. The time specified in step 6 is for guidance only and should be optimized by the user based on the reaction volume. Larger reaction volumes may require a longer duration. For instance, with a 400 µl reaction volume, it is recommended to extend the time at step 6 to 54 hours.

Reconstitution

1. For reconstitution, it is advisable to use the same volume of DNA sample as was the total reaction volume before lyophilization. For example, if the lyophilized reaction volume was 20 µl, it is recommended to reconstitute the lyophilizate with 20 µl of DNA sample. Further optimization of sample volume may be necessary to reach a suitable reaction volume.

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

DS-28-52 v1

Effective from: 10.01.2024

*__Product stability__ is assessed using routine QC assays and QC criteria set forth in the product specification and are intended to provide guidelines for shipping and storage conditions only. Customer or its designee shall be responsible for conducting all necessary stability testing applicable to their assay and/or QC criteria, and to comply with any applicable regulatory requirements or guidelines. Such stability testing shall include testing to validate the lead times for shipment, the shelf life of, and the product specifications applicable to shipment, storage and handling of the assay assembled and packed by the customer.

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