SOLIScript® Lyo-ready 1-step RT-qPCR Kit

Catalogue Number	Pack Size
08-92-0000S	100 x 20 µl reactions
08-92-00250	250 x 20 µl reactions
08-92-02000	2000 x 20 µl reactions
08-92-05000	5000 x 20 µl reactions

Store at –20°C

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
- Can be stored at 4°C (2°C to 8°C) for up to 3 months
- Stability at room temperature (15–25°C) for 2 weeks
- Freeze-thaw stability: 10 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:

- **SOLIScript® Lyo-ready 1-step RT-qPCR Kit** is an optimized real-time one-step (RT-qPCR) solution compatible with lyophilization. The kit enables sensitive and accurate quantification of RNA and DNA targets using dual-labeled hydrolysis probes (e.g. TaqMan® probes), and is suitable for detection and quantitation of up to five targets simultaneously. The critical temperature (Tc) of the kit is -30 °C and the glass transition temperature (Tg) of the lyophilized product is 85.9 °C. The moisture content of the lyophilized product is 0.92%.
- The Kit comes in 4 tubes and contains all the components necessary (except water, RNA template, probe(s), and primers) to perform RT-qPCR in a wet or lyophilized format.
- **40x SOLIScript® Lyo-compatible One-Step Mix** is a 40x concentrated RT mix that includes an *in silico* designed SOLIScript® reverse transcriptase and RiboGrip® RNase Inhibitor. SOLIScript® is a thermostable reverse transcriptase active at temperatures up to 60°C, beneficial when using templates with high levels of secondary structure. RiboGrip® inactivates RNase A to protect RNA sample from degradation and increase cDNA yield in reactions with low RNA amounts.
- **HOT SolisFAST® Glycerol-Free DNA Polymerase (10 U/µl)** is an *in silico* designed inhibitor tolerant analogue of *Taq* DNA polymerase with chemical hot-start and ~2-4 times faster extension rates compared to the wild-type *Taq*.
- **4x Lyo Reaction Buffer** contains additives for efficient amplification. The $MgCl_2$ concentration is 20 mM (1x mix – 5 mM).
- **4x RT-qPCR 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 both cakes and beads.
- The Kit can be used for gene expression analysis and low-copy gene detection, gene knockdown validation, miRNA profiling and quantification, characterization of GMOs, RNA viral pathogen detection and quantification.

Content:

Kit Component	Product sizes (for 20 µl rxn) and volumes			
	100 rxn	250 rxn	2000 rxn	5000 rxn
40x SOLIScript® Lyo-compatible One-Step Mix	50 µl	125 µl	1 ml	2.5 ml
HOT SolisFAST® Glycerol-Free DNA Polymerase (10 U/µl)	40 µl	100 µl	0.8 ml	2 ml
4x Lyo Reaction Buffer	500 µl	1250 µl	10 ml	25 ml
4x RT-qPCR Lyo Excipient Mix	500 µl	1250 µl	10 ml	25 ml

Note: To avoid repeated freezing and thawing as well as to minimize the contamination risk of stock solutions of reagents, it is highly recommended to divide large-volume stocks into several smaller aliquots and store them at -20°C.

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 RNA sample.
2. Thaw 40x SOLIScript® Lyo-compatible One-Step Mix, HOT SolisFAST® Glycerol-Free DNA Polymerase (10 U/µl), 4x Lyo Reaction Buffer, 4x RT-qPCR 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	Up to 20 µl	
2	Forward Primer(s) (10 µM)[2]	0.6 µl	300 nM each
3	Reverse Primer(s) (10 µM)[2]	0.6 µl	300 nM each
4	Probe(s) (10 µM)[2]	0.2 µl	100 nM each
5	4x Lyo Reaction Buffer	5 µl	1x
6	HOT SolisFAST® Glycerol-Free DNA Polymerase (10 U/µl)	0.4 µl	1x
7	40x SOLIScript® Lyo-compatible One-Step Mix	0.5 µl	1x
8	4x RT-qPCR Lyo Excipient Mix	5 µl	1x
9	Template DNA	Variable	Variable
Total		**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 300 nM each primer and 100 nM probe are suitable for most applications.

5. Run the RT-qPCR reactions immediately after reaction setup. Follow the cycling instructions on page 7.

Step-by-step guidelines for lyophilization:

1. Thaw 40x SOLIScript® Lyo-compatible One-Step Mix, HOT SolisFAST® Glycerol-Free DNA Polymerase (10 U/µl), 4x Lyo Reaction Buffer, 4x RT-qPCR 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 you are lyophilizing without primers and probes, replace the respective volume with water.

Step	Component	Volume[1]	Final conc.
1	Nuclease-free water	Up to 20 µl	
2	Forward Primer(s) (10 µM)[2]	0.6 µl	300 nM each
3	Reverse Primer(s) (10 µM)[2]	0.6 µl	300 nM each
4	Probe(s) (10 µM)[2]	0.2 µl	100 nM each
5	4x Lyo Reaction Buffer	5 µl	1x
6	HOT SolisFAST® Glycerol-Free DNA Polymerase (10 U/µl)	0.4 µl	1x
7	40x SOLIScript® Lyo-compatible One-Step Mix	0.5 µl	1x
8	4x RT-qPCR Lyo Excipient Mix	5 µl	1x
Total		**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 °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	100 min	Atmospheric	Ramp (0.5 °C/min)
3	-45 °C	180 min	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	+20 °C	120 min	80 µbar	Ramp (0.5 °C/min)
8	+20 °C	360 min	80 µbar	Hold
STOPPERING STAGE				
9	+5 °C	n/a	Atmospheric	Backfill with N_2
10	+5 °C	n/a	Atmospheric	Open the door and stopper
11	+5 °C	n/a	Atmospheric	Stop N_2 flow

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.

Recommended cycling protocol			
Step	**Temperature**	**Time**	**Cycles**
Reverse transcription	50°C	10 min	1
Initial denaturation[1]	95°C	10 min	1
Denaturation[2]	95°C	1-5 sec	40
Annealing/extension[2]	60–65°C	5-20 sec	

[1] Enzyme activation step at 95°C for 10 min is crucial for full activation of DNA polymerase and inactivation of reverse transcriptase.

[2] 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

Lyophilization

1. The time specified in step 6 of lyophilization protocol 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

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This product is supplied for research use only. It is suitable for use as a component of molecular diagnostic assays, where applicable country laws allow. This product alone does not provide any diagnostic result. Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.

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