SolisFAST® Direct qPCR Kit (ROX), 5x

Catalogue Number	Pack Size	20 µL rxn
28-06-0000S	0.2 mL	50
28-06-00250	1 mL	250
28-06-01250	5 x 1 mL	1250
28-06-05000	20 mL	5000

Store at -20 °C

Shipping:

At room temperature

Batch Number and Expiry Date:

See vial

Storage and Stability:

- Routine storage at -20 °C until expiry date
- The Kit 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 ISO 9001 and ISO 13485 certified Quality Management System.

Product description:

- SolisFAST® Direct qPCR Kit (ROX) is a 5x concentrated, ready-to-use solution optimized for high inhibitor tolerance, direct sample applications, and probe-based 5-plex qPCR assays, allowing sample input volumes of up to 50% of the total reaction volume while maintaining robust and reliable amplification performance.
- 10x Lysis Buffer is a universal lysis buffer in 10x concentration that is designed for rapid DNA extraction from wide range of biological samples, including human buccal swabs, mouse biopsies, crop seeds, and plant leaves. The resulting crude lysates can be used directly in downstream qPCR applications, eliminating the need for conventional DNA purification.
- The kit contains all components necessary, except primers, probe(s), nuclease-free water, and DNA template.
- The kit is optimized for dual-labeled hydrolysis probes (e.g., TaqMan® probes) and is suitable for detection and quantitation of up to five targets simultaneously.

Kit Contents:

Kit components	Catalogue Number			
	28-06-0000S	28-06-00250	28-06-01250	28-06-05000
5x SolisFAST® Direct qPCR Mix (ROX)	0.2 mL	1 mL	5 x 1 mL	20 mL
10x Lysis Buffer	0.5 mL	2.5 mL	5 x 2.5 mL	50 mL

5x SolisFAST® Direct qPCR Mix (ROX) components:

Mix components	Description
SolisFAST® DNA polymerase	An *in silico*-designed variant of *Taq* DNA polymerase featuring enhanced room-temperature stability through a genetically engineered **Stability TAG***, rapid hot-start activation, and faster extension rates compared to wild-type *Taq* DNA polymerase.
qPCR buffer	Contains $MgCl_2$, dNTPs (dATP, dCTP, dGTP, and dTTP), and additives optimized to enhance PCR amplification efficiency.

Compatible real-time instruments:

The mix is compatible with qPCR instruments that require ROX as a passive reference dye for fluorescence signal normalization. Scan the QR code for detailed information and a list of compatible instruments.

Before you begin:

Dilute the 10x Lysis Buffer to a 1x concentration with PCR-grade water to produce the required volume of 1x Lysis Buffer, which is approximately 100 µl of 1x Lysis Buffer per sample.[1]

Select the appropriate sample preparation procedure based on the sample type.

Plant sample preparation

1. Prepare a plant tissue sample smaller than < 4 mm in diameter (per 100 µl of 1x Lysis Buffer) or break plant seeds to a size of 1-2 mm.

2. Add 50–150 µL of 1x Lysis Buffer to the microcentrifuge tube or 96-well PCR plate containing the sample material prepared in the previous step. Keep the tubes or plate on ice or a cooling block during preparation.[2]

3. Ensure the sample is fully submerged in 1x Lysis Buffer and incubate at 25 °C for 3 min using an incubator or (q)PCR instrument.

4. Briefly vortex the tube, centrifuge at 2,000 rpm for 1 min at room temperature (RT) or 4 °C, and place it on a cooling block. If not used immediately for PCR, store the lysate at -20 °C

5. Use 1-5 µL of the supernatant for a 10 µL qPCR assay or 1-10 µL of the supernatant for a 20 µL qPCR assay.[3]

[1] See **Table 1** below for recommended amounts per sample. 150 µL of 1x Lysis Buffer is recommended in case of high carbohydrate content.

[2] It is highly recommended to optimize the lysis mix/sample ratio for optimal performance in PCR, as different sample materials (for instance fresh leaves/seeds) have different optimums.

[3] It is recommended to keep the lysate on ice or at 4 °C while assembling the reaction mix.

Table 1. Recommended amount of 1x Lysis Buffer per sample depending on the sample type and size. The final amount should be optimized for the exact setup of the experiment (50-150 µl for seeds and 25-100 µl for leaves).

Sample type	Seed sample (1-2 mm diam.)	Leaf sample (1-2 mm in diam.)	Leaf sample (2-4 mm in diam.)
Dry/lyophilized	100 µl	25 µl	50 µl
Fresh	100 µl	50 µl	100 µl

Preparation of blood and liquid samples

1. Transfer 2 µL of the blood[4] or liquid sample to a tube containing 100 µL of 1x Direct Extraction Buffer.[5]
2. Close the tube and vortex briefly for 15 seconds.
3. Incubate at room temperature (20–25 °C) for 2–3 minutes.
4. Use 1–5 µL of the supernatant for a 10 µL qPCR assay or 1–10 µL for a 20 µL qPCR assay.[3]

[4] The protocol has been successfully tested using 1 µL of whole blood added directly to the qPCR reaction mixture of 10 µL, eliminating the need for sample lysis (steps 1–3).

[5] A sample-to-buffer dilution ratio of 1:50 to 1:100 is recommended. The optimal dilution ratio may vary depending on the sample type and assay conditions.

Preparation of nasal and throat swab samples

1. Add 200 µL of 1x Direct Extraction Buffer to a 1.5 mL microcentrifuge tube.
2. Cut off the swab tip containing the collected sample and place it into the tube.
3. Close the tube and vortex for 15 seconds.
4. Incubate at room temperature (20–25 °C) for 2–3 minutes.
5. Remove the swab tip and squeeze it against the inner wall of the tube to recover the remaining liquid.
6. Briefly centrifuge the tube and use 1–5 µL of the supernatant for a 5 µL qPCR assay or 1-10 µL for a 20 µL qPCR assay.[3]

Preparation of animal tissue samples

1. Add 100 µl of 1x Lysis Buffer to each sample of 4 mm.[6]
2. Incubate on the thermal block for 3 minutes at 25 °C. Incubate at room temperature (20–25 °C) for 2–3 minutes.
3. Briefly vortex the tube, centrifuge at 2,000 rpm for 1 min at room temperature (RT), and place it on a cooling block. If not used immediately for PCR, store the lysate at -20 °C.
4. Use 1-5 µL of the supernatant for a 10 µL qPCR assay or 1-10 µL of the supernatant for a 20 µL qPCR assay. [3]

[6] The following samples have been successfully tested: mouse ear- and tail tags of 4 mm. The optimal sample size and lysis buffer volume depend on the sample type and assay conditions. Therefore, the sample-to-lysis buffer ratio should be optimized for each new assay to ensure optimal PCR performance, as different sample materials (e.g., blood and epidermal tissue) may require different ratios.

Step-by-step guidelines for consecutive qPCR:

1. Thaw 5x SolisFAST® Direct qPCR Mix (ROX), template DNA, primers, probe(s), and nuclease-free water. Gently mix each component by vortexing or pipetting up and down, then briefly centrifuge.
2. Prepare the reaction mix by combining all required components except the template DNA.

Component	Volume[A]	Volume[A]	Final conc.
5x SolisFAST® Direct qPCR Mix (ROX)	2 µL	4 µL	1X
Forward Primer (10 µM)	0.4 µL	0.8 µL	400 nM[B]
Reverse Primer (10 µM)	0.4 µL	0.8 µL	400 nM[B]
Probe(s)	-	-	200 nM[C]
Template DNA (added at step 4)	Variable	Variable	-
Nuclease-free water	Up to 10 µL	up to 20 µL	-
Total reaction volume	**10 µL**	**20 µL**	-

[A] Scale all components proportionally based on the number of reactions and the reaction volume. Prepare the reaction mix with an additional 10% volume to compensate for pipetting losses.

[B] The final primer concentration may be adjusted within the range of 300–800 nM, with 400 nM recommended as the starting concentration.

[C] The final probe concentration may be adjusted within the range of 100–400 nM, with 200 nM recommended as the starting concentration.

3. Mix the reaction mix thoroughly, briefly centrifuge, and dispense the appropriate volume into each PCR well.
4. Add the appropriate volume of DNA-containing supernatant to each PCR well.
5. Seal the wells according to the thermal cycler manufacturer's instructions, then centrifuge the reactions at 2,000 rpm for 2 min at RT.
6. Program the thermal cycler according to the recommended cycling conditions provided below.

Step	Temperature	Time	Cycles
Enzyme activation and initial denaturation	95 °C	30 sec-2 min[D]	1
Denaturation	95 °C	1-5 sec[E]	40
Annealing/extension	60-65 °C	5-20 sec[E]	

[D] An initial incubation of 30 sec up to 2 minutes is sufficient for enzyme activation. An initial denaturation step of 2 min is recommended to ensure complete DNA denaturation.

[E] The cycling program may be optimized based on the thermal cycler specifications, assay design, and desired run time. The optimal annealing/extension temperature depends on the melting temperatures (T_m) of the primers and probe. Gradient PCR is recommended to determine the optimal annealing/extension temperature for each assay.

7. Place the reactions into the qPCR cycler, start the qPCR run.

Recommendations for a successful qPCR experiment

Primer design:

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

1. For optimal qPCR amplification efficiency, design primers that generate amplicons between 50 and 150 bp in length.
2. The recommended primer length is 18-24 nucleotides
3. Avoid primer sequences containing stretches of more than four consecutive G and/or C nucleotides.
4. The recommended primer GC content is 35-65%. Primers with higher GC content may require additional optimization, such as the addition of DMSO, betaine, or other PCR-enhancing additives, to improve amplification performance.
5. The recommended melting temperature (T_m) for primers is approximately 60 °C, with a T_m difference of no more than 3 °C between the forward and reverse primers to ensure efficient annealing. Probes should be designed with a T_m approximately 10 °C higher than that of the primers to promote stable hybridization during amplification.
6. Evaluate primer sequences for self-complementarity, particularly at the 3' ends, to minimize the risk of primer-dimer formation.
7. A final primer concentration of 400 nM and a probe concentration of 200 nM are recommended for most qPCR assays. If amplification efficiency is suboptimal, optimize the primer concentration by performing a titration within the range of 100-600 nM.

DNA template:

The integrity, purity, and concentration of DNA templates should be suitable for qPCR analysis.

1. The optimal DNA template input for qPCR depends on the template type, quality, and target abundance.
2. The recommended DNA sample input is up to half of the total qPCR reaction volume. Higher input volumes may compromise amplification efficiency due to residual reverse transcription reagents or other components that may inhibit qPCR.
3. Perform qPCR reactions in triplicate using a serially diluted template (e.g., a 10-fold dilution series) and analyze the resulting amplification data.
4. Prepare fresh serial dilutions in deionized water before each experiment. Generate a standard curve from the dilution series to assess qPCR amplification efficiency and determine the optimal template concentration for your assay.
5. Include a no-template control (NTC) in each qPCR experiment to monitor potential contamination and nonspecific amplification, including primer-dimer formation. Prepare the NTC by replacing the DNA template with nuclease-free water.

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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***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. The 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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