SolisAcura™ Probe Genotyping qPCR Mix (no ROX), 5x

Catalogue Number	20 µl rxn	Pack Size
38-01-0000S	50	0.2 ml
38-01-00250	250	1 ml
38-01-00250-5	5 x 250	5 x 1 ml
38-01-05000	5000	20 ml

Store at -20 °C

Stability at room temperature 1 MONTH

Shipping:

At room temperature

Batch Number and Expiry Date:

See vial

Storage and Stability:

- Routine storage at -20 °C (–28°C to –18°C) until Expiry Date
- Stability at room temperature (15 – 25 °C) for 1 month
- Freeze-thaw stability: 10 cycles

Reaction setup:

At room temperature

Manufactured by Solis BioDyne, in compliance with ISO 9001 and ISO 13485 certified Quality Management System.

Product description:

- SolisAcura™ Probe Genotyping qPCR Mix (no ROX) is a 5X concentrated, ready-to-use SNP genotyping solution optimized for fast, highly sensitive and reproducible probe-based qPCR assays using dual-labeled hydrolysis (e.g. TaqMan®), hybridization or displacement probes.
- SolisAcura™ Exo(+) DNA Polymerase is modified to be extremely sensitive to mismatches on the 3' ends of primers.
- The mix is suitable for detection and quantification of up to four targets simultaneously.
- The mix contains all components necessary, except primers, nuclease-free water, and DNA template.

Mix component	Description
SolisAcura™ Exo(+) DNA Polymerase	*In-silico* designed analogue of *Taq* DNA polymerase with enhanced stability at room temperature, fast oligo-based hot-start, enhanced mismatch discrimination, higher fidelity and faster extension rates compared to the wild-type *Taq* DNA polymerase
qPCR buffer	Includes 15 mM $MgCl_2$ (1x PCR solution 3 mM $MgCl_2$), dNTPs (dATP, dCTP, dGTP, dTTP), stabilizers and enhancers that maximize efficiency of PCR reaction

Compatible real-time instruments:

As endpoint signals from multiple channels are used to carry out the clustering by genotype, the mix is compatible with endpoint PCR cyclers and qPCR cyclers that do not require an internal reference dye (e.g., ROX) for normalization of fluorescent signal. For clustering-based genotyping, endpoint PCR cycler experiments can be measured on a plate reader.

Step-by-step guidelines:

1. Thaw SolisAcura™ Probe Genotyping qPCR Mix, template DNA, primers, 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.

Component	Volume[1]			Final conc.
SolisAcura™ Probe Genotyping qPCR Mix (no ROX) (5X)	4 µl	2 µl	1 µl	1X
Forward Primer (10 µM)[2]	0.6 - 1.8 µl	0.3 – 0.9 µl	0.15 – 0.45 µl	300-900 nM (each)
Reverse Primer (10 µM)[2]	0.6 – 1.8 µl	0.3 – 0.9 µl	0.15 – 0.45 µl	300-900 nM (each)
Probe(s)[2]	Variable	Variable	Variable	100-300 nM (each)
Template DNA (added at step 4)	Variable	Variable	Variable	<10 ng/µl
Nuclease-free water	up to 20 µl	up to 10 µl	up to 5 µl	
Total reaction volume[3]	**20 µl**	**10 µl**	**5 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 300-900 nM, and probe concentration between 100-300 nM. A final concentration of 400 nM each primer and 150 nM probe is suitable for most applications. The primer concentrations do not have to be equal.

[3] Total reaction volume of 5-20 µl has been tested. The final concentrations of the components should remain the same as in the table.

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 procedure recommended for the cycling instrument being used, centrifuge the reactions briefly.

6. Program the thermal cycler using the cycling conditions recommended below.

Step	Temperature	Time	Cycles
Plate pre-read[1] (qPCR cycler or plate reader; OPTIONAL)	58 – 65 °C	various	1
Enzyme activation[2]	95 °C	3 min	1
Denaturation[3]	95 °C	10 sec	40
Annealing/extension[3]	58 – 65 °C	30 sec	
Plate post-read[4] (qPCR cycler or plate reader; OPTIONAL)	58 – 65 °C	various	1

[1] Pre-read step is optional for measuring varying autofluorescence coming from different sample types (purified versus crude). This step is recommended with small sample amounts and clustering-based genotyping.

[2] With low-complexity templates (e.g., cDNA), shorter initial denaturation time (30-60 sec) 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 between 1-10 sec and annealing/extension time between 5-30 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.

[4] The post-read step is required for clustering-based genotyping but can be omitted for experiments that do not involve clustering.

7. Place the reactions into the qPCR cycler, start the qPCR run.
8. After the reaction is completed, perform data analysis according to the instrument-specific instructions.
9. Should the samples not form well-defined clusters after completion of the PCR program, additional cycling is recommended to allow all samples to reach amplification plateau. Recommended cycling conditions is 3 additional cycles of denaturation and annealing/extension with the optimal temperatures determined for the specific experiment, followed by an optional plate read step.

Recommendations for a successful qPCR experiment

For accurate genotype assignment and clustering, the experiment is recommended to be carried out with **2 no-template-control samples (NTCs)**, where DNA is replaced with nuclease-free water (Cat no. water-025).

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.

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

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

Primers:

1. The optimal primer length is 20-30 base pairs targeting amplicons of 70 to 150 base pairs. Avoid runs of more than four consecutive G or C bases.

2. GC-content should range from 35 – 65 %. Higher GC-content may need more optimization by adding DMSO, betaine or other additives to improve the results.

3. The optimal melting temperature (T_m) of the primers is 60-65°C. For both primers to bind efficiently, the T_m of the two primers should not differ by more than 3 °C. For Tm calculations, use NCBI Blast or Primer3 software.

4. Analyze your PCR primers for self-complementarity in their sequences. Avoid the 3'-self complementarity, because it increases possibility of primer-dimers formation.

5. A final primer concentration of 0.4 µM is suitable for most PCR conditions. If your primers do not amplify efficiently, determine an optimal primer concentration using primer titration in the range of 0.3 – 0.9 µM.

Probes:

1. Although dual-labeled probes with BHQ® have been tested, MGB-type probes are preferred due to their greater robustness and sensitivity.

2. Probe concentrations can be symmetrical or asymmetrical.

Template:

The integrity, purity and concentration of 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/or heat treatment and to optimize the sample volume per reaction.

2. The recommended final concentration of DNA template for qPCR experiment is dependent upon the type of DNA used. For example, if using cDNA as a template, qPCR efficiency would be largely dependent on the expression level of target gene. Use up to 10 ng/µl of complex (e.g. eukaryotic) DNA or cDNA.

3. Recommended final amount of cDNA sample in qPCR reaction mixture is up to one tenth of the final reaction volume. Overloading the cDNA sample may inhibit the reaction.

4. Perform and analyze your qPCR reactions in triplicates on a serially diluted template (e.g. 10-fold dilution series). Use standard curve derived from serial dilutions to assess qPCR efficiency and to determine the optimal template concentration for your 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.

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. 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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