HOT SolisAcura™ Cassette-Based Genotyping Mix (ROX), 2x

Catalogue Number	10 µl rxn	Pack Size
38-12-0000S	50	0.25 ml
38-12-00200	200	1 ml
38-12-00200-5	1000	5 x 1 ml
38-12-05000	5000	25 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.
- Stable at room temperature (25°C) for 1 month.
- 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:

- HOT SolisAcura™ Cassette-Based Genotyping Mix (ROX) is a 2X-concentrated, ready-to-use SNP genotyping solution optimized for highly sensitive and reproducible cassette-based assays using FRET cassettes. It is similar in principle to KASP™ (Kompetitive Allele Specific PCR) and is fully compatible with existing KASP™ assays, as well as PACE® and Amplifluor® methods.
- The mix contains all components necessary, except primers, nuclease-free water, and DNA template.
- It is suitable for SNP detection via allele-specific PCR, using bi-allelic discrimination that is achieved with a universal reverse primer and two competitively binding allele-specific forward primers, each with a unique tail sequence that corresponds to either of two universal FRET cassettes.
- HOT SolisAcura™ Exo(-) DNA Polymerase used in this cassette-based mix is a chemically modified DNA polymerase, enabling hot-start PCR that improves specificity and accuracy, minimizes mispriming and extension from non-specifically annealed primers and primer-dimers. The DNA polymerase in this cassette-based mix is inactive at room temperature and is activated by an initial activation step for 15 min at 95°C.
- HOT SolisAcura™ Exo(-) DNA Polymerase is extremely sensitive to mismatches on the 3' ends of primers, resulting in superior performance in SNP detection and allele discrimination. The novel polymerase is also engineered to have faster synthesis rates and is inherently inhibitor tolerant ensuring excellent performance with crude or challenging samples. The faster synthesis rates allow for faster cycling protocol as well, however it is dependent on the technical apparatus.

Mix component	Description
HOT SolisAcura™ Exo(-) DNA Polymerase	*In-silico* designed analogue of *Taq* DNA polymerase that is sensitive and with high processivity and inhibitor tolerance making it perfect for SNP detection. It has enhanced stability at room temperature, chemical hot-start, enhanced

	mismatch discrimination, higher fidelity and faster extension rates compared to the wild-type *Taq* DNA polymerase.
Reaction buffer with cassettes	Includes 5.6 mM $MgCl_2$ (final concentration 2.8 mM $MgCl_2$), dNTPs (dATP, dCTP, dGTP, dTTP), stabilizers and enhancers that maximize efficiency of the reaction. Two fluorescently labelled FRET cassettes (allele 1 – FAM channel, allele 2 – HEX channel). Low ROX.

Step-by-step guidelines:

1. Thaw the reagents at room temperature. Mix each reagent by gentle vortexing or pipetting up and down, then spin down.

2. Prepare a reaction mix at room temperature. Add all required components except the template DNA.

Component	Volume[1]	Final conc.
HOT SolisAcura™ Cassette-Based Genotyping Mix (ROX) (2X)	5 µl	1x
Primer mix[2] Allele-specific forward primer 1 (12 µM), Allele-specific forward primer 2 (12 µM), Common reverse primer (30 µM)	0.14 µl	Forward primers 0.165 µM each, Reverse primer 0.420 µM
Template DNA (added at step 4)	Variable	1-50 ng
Nuclease-free water	up to 10 µl	
Total reaction volume	**10 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 primer concentrations depend on the specific primers and may require empirical evaluation.

NB! Please note that the template specific forward primers and reverse primer are not included in the kit and should be provided by the user. However, as the kit is compatible with the KASP™, PACE® and Amplifluor® methods, any primer assays designed for these would be compatible.

3. Mix the reaction mix thoroughly, then centrifuge briefly. Dispense appropriate volumes of mix into PCR wells or tubes.

4. Add template DNA to the PCR wells. Seal the wells using the procedure recommended for the cycling instrument being used and centrifuge the reactions briefly.

5. Program the thermal cycler using the standard cycling conditions recommended **below**. If you would prefer to use a faster cycling protocol due to the specifics of the technical apparatus used, you may refer to our technical support (support@solisbiodyne.com).

Step	Temperature	Time	Cycles
Initial activation[1]	95°C	15 min	1
Touchdown PCR			
Denaturation[2]	95°C	20 sec	10
Annealing/extension (decreasing 0.8°C per cycle)[2]	65-57°C	60 sec	
Final cycling			
Denaturation[2]	95°C	10 sec	36
Annealing/extension[2]	59°C	60 sec	
Plate read[3] (qPCR cycler or plate reader)	35°C	various	1

[1] Initial incubation at 95 °C for 15 min is needed for the activation of polymerase and denaturation of template DNA.

[2] The cycling program can be optimized depending on the instrument specification, assay design and the desired total run time. Denaturation between 10-20 sec and annealing/extension time between 20-60 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.

[3] Due to the nature of the genotyping assay, it is crucial to cool the samples to ≤ 40°C before reading the plate and analysing genotypes. If a plate reader is used for signal acquisition, it is not necessary to add this step to the PCR program.

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

7. After the reaction is completed, perform data analysis according to the instrument-specific instructions.

8. 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.

Plate settings

The detection of amplified fragments is performed in the following channels: FAM (allele 1), VIC/HEX/JOE (allele 2), Cal Red 610/ROX/Texas Red (ROX reference dye).

Recommendations for a successful PCR 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.

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.

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, 10x GC-rich enhancer (Cat no. 05-16-00010) or other additives to improve the results.

3. For all three primers to bind efficiently, the T_m of the three 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. Additionally, Solis BioDyne can confirm the compatibility of existing primers, should you be willing to share their sequences. In this case, you may write to our technical support (support@solisbiodyne.com).

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.

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.

Troubleshooting Guide

No or low PCR yield

- HOT SolisAcura™ Exo(-) DNA Polymerase was not activated and/or template DNA was not fully denatured – make sure that your PCR starts with an initial incubation at 95°C for 15 minutes.
- Cycling conditions are not optimal – adjust touchdown PCR annealing/extension temperature by increasing or decreasing it by 1-2°C and optimising the final cycling's annealing/extension temperature accordingly; increase the number of final cycling phase's cycles by 3–5.
- Poor quality of template – check the template's purity and integrity, ensure that your template doesn't contain PCR inhibitors. You can also dilute the DNA, if a lot of inhibitors are present.
- Template concentration is too low – increase the concentration of DNA template.
- Reaction components are degraded – check the storage conditions and expiry date of the reagents; perform a positive control with template DNA and/or reagents previously known to amplify.

Non-specific products

- Non-specific amplification – ensure that your primers are target-specific.
- Primer annealing temperature is too low – increase the annealing temperature; keep your primer annealing temperature 2–5°C below the T_m of the primer having the lowest T_m.
- Too many cycles – reduce the final cycling phase's cycle number by 3–5 to remove non-specific bands.
- Too little cycles – increase the final cycling phase's cycle number by 3 to ensure the samples reach amplification plateau.

Safety precautions:

Please refer to the 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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