

HOT SolisAcura™ Exo(-) DNA Polymerase Kit

Catalogue Number	Pack Size (10 U/μl)
01-18-KIT-0000S	250 U
01-18-KIT-00500	500 U
01-18-KIT-01000	1000 U



Shipping:

At room temperature.

Store at -20 °C

Batch Number and Expiry Date:

See vial.

**Stability at room
temperature
1 MONTH**

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™ Exo(-) DNA Polymerase has been genetically modified to be 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.
- HOT SolisAcura™ Exo(-) DNA Polymerase has chemical hot-start PCR that improves specificity and accuracy, minimizes mispriming and extension from non-specifically annealed primers and primer-dimers. The enzyme is inactive at room temperature and is activated by an initial activation step for 15 min at 95°C.
- This enzyme 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 possesses 5'→3' polymerase activity, however no 3'→5' exonuclease (proofreading) activity and no 5'→3' exonuclease activity.

Kit Content:

Component	Catalogue Number		
	01-18-KIT-0000S	01-18-KIT-00500	01-18-KIT-01000
HOT SolisAcura™ Exo(-) DNA Polymerase (10 U/μl)	250 U / 25 μl	500 U / 50 μl	1000 U / 100 μl
10x Reaction Buffer C	0.5 ml	0.5 ml	0.5 ml
25 mM MgCl ₂	0.5 ml	0.5 ml	0.5 ml

Additional reagents required:

- Template DNA
- Gene-specific primer pair
- dNTP Mix (20 mM of each, Cat. No. 02-31-00020)
- Nuclease-free PCR Grade Water (Cat. No. water-025)

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™ Exo(-) DNA Polymerase (10 U/ µl)	0.20 - 0.48 µl	0.1 - 0.24 U/µl
10x Reaction Buffer C	2 µl	1x
dNTP Mix (20 mM of each)	0.25 µl	250 µM of each
Forward Primer (10 µM) ²	0.4-0.8 µl	200-400 nM
Reverse Primer (10 µM) ²	0.4-0.8 µl	200-400 nM
25 mM MgCl ₂	1.76 - 2.24 µl	2.2 - 2.8 mM
Template DNA (added at step 4)	Variable ⁴	Variable ⁴
Nuclease-free water	up to 20 µl	
Total reaction volume	20 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 template and may require optimization. For multiplex amplification, the final concentration of individual primers should be reduced to 100–200 nM.

⁴ Polymerase works well with 20 fg – 100 ng of DNA per 20 µl reaction, depending on the complexity of the template.

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 cycling conditions recommended below.

Step	Temperature	Time	Cycles
Initial activation ¹	95°C	15 min	1
Denaturation	95°C	5 sec	25–35
Annealing/extension ²	55–65°C	20 sec	

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

² The annealing temperature depends on the melting temperature of the primers. Annealing/extension time depends on the length of the fragment to be amplified. Extension time is 15–30 sec/kb, depending on the target template.

Recommendations for a successful PCR experiment

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. The optimal melting temperature (T_m) of the primers should be between 55-65°C. For all primers to bind efficiently, the T_m of the two primers should not differ by more than 3°C. For T_m 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.

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 (10 U/μl) was not activated – make sure that your PCR starts with an initial incubation for 15 min at 95 °C.
- Cycling conditions are not optimal – adjust annealing temperature; if needed determine the optimal annealing temperature by running a temperature gradient; adjust denaturation temperature and time; increase the extension time (if amplifying a long target); increase the number of cycles by 3–5.
- Poor quality of template – check the template's purity and integrity, ensure that your template doesn't contain PCR inhibitors.
- Template concentration is too low – increase the concentration of DNA template.
- Primer concentration is not optimal – titrate primer concentration (final concentration 200-400 nM of each); ensure that both primers have the same concentration.
- 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 concentration is not optimal – titrate primers (final concentration 200-400 nM of each); too high primer concentration can reduce the binding specificity, resulting in unwanted products.
- 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 cycle number by 3–5.

- Too little cycles – increase the cycle number by 3 to ensure the samples reach amplification plateau.
- Contamination – to avoid contamination, work in dedicated space, keep pre- and post-amplification areas separate, use personal protective equipment, decontaminate your surfaces and equipment, if possible, aliquot your reagents into smaller volumes to prevent contamination of stock solutions.

Smearing in electrophoresis

- Too much template – load lower amount or prepare serial dilutions of template.
- Too many cycles – reduce the cycle number by 3–5.
- Extension time is too long – reduce extension time.
- Primer design is not optimal – review your primers and redesign the primers if needed.

Unit definition:

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 74°C.

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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****Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.**

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