SHARP
Diagnostics

SHARP

Isothermal Amplification Kit

Instruction Manual

For research use only

Overview

SHARP is an isothermal DNA amplification method that uses a bioengineered super helicase, single-stranded DNA-binding protein (SSB), and a strand-displacing DNA polymerase to achieve rapid DNA amplification at a constant temperature. Reactions are assembled with standard PCR primers and routine molecular biology reagents, allowing SHARP to integrate readily into existing laboratory workflows without the need for thermocycling.

This instruction manual describes the use of the **SHARP Isothermal Amplification Kit**. The amplification kit provides all components required to perform **200 x 20 µL SHARP reactions**. The reagents are formulated for convenience so that, in the reference 20 µL reaction, 2 µL of 10X Reaction Buffer and 1 µL of each remaining component reproduce SHARP Diagnostics' internal reference condition for amplification of the positive control amplicon. Under proper handling and recommended running conditions, the positive control reaction should exhibit the following performance characteristics:

- Amplification of a 198 bp segment of lambda phage DNA (segment 43,956–44,153)
- Detectable amplification typically observed in under 10 minutes at 65 °C
- Completion of amplification between 15-20 minutes.

Like all DNA amplification systems, SHARP reaction performance depends on primer design, template, and reaction conditions. The formulations provided in this manual are suitable as a general starting point, and the individual components are supplied separately to allow users to adjust enzyme ratios, primer concentrations, ATP, and buffer conditions as needed to optimize SHARP for specific applications.

Support and User Feedback

We are actively refining SHARP and consider every use case an important part of that process. If you have questions about troubleshooting or optimizing SHARP for your specific application, please contact us at **SUPPORT@SHARPDX.COM**. Even if your experiments run smoothly, we greatly appreciate hearing what worked well, what did not, and how SHARP fit into your workflow. Your experience directly informs how we improve the chemistry, documentation, and future kit designs, and we view every user as a partner in shaping the SHARP platform.

Materials Provided

SHARP Isothermal Amplification Kit (200 reactions)

Component	Volume Provided
SHARP Reaction Buffer* (10X)	400 µL
dNTP Mix (10 mM each)	200 µL
ATP (100 mM)	200 µL
PcrA-M6 Helicase (20X)	200 µL
SSB Protein (20X)	200 µL
Bst-LF Polymerase (20X)	200 µL

*1.0 mg/mL BSA, 500 mM potassium acetate, 100 mM magnesium acetate, 200 mM tris-acetate, pH 7.9

SHARP Positive Control Kit (100 reactions)

Component	Volume Provided
λ-DNA Template (5 ng/µL)	100 µL
λ 200 Primers (20 µM each)	100 µL

Additional Materials Required

Users will need to supply the following items to perform SHARP reactions:

- Nuclease-free water
- Purified DNA template
- Forward and reverse primers
- **Optional:** DNA-binding dye (e.g. EvaGreen/SYBR Green) for real-time monitoring

Storage Conditions (Important)

The **SHARP Isothermal Amplification Kit** and the **SHARP Positive Control Kit** should be stored at –20 °C. The enzyme components (PcrA-M6 helicase, SSB, and Bst-LF polymerase) and the 20x reaction buffer do not freeze at this temperature. All other components will freeze solid at –20 °C.

ATP and **SSB** degrade over time and with repeated freeze thaw cycles. For long-term storage, we recommend **aliquoting** these components upon receipt and storing at **–80°C**, keeping one working aliquot at –20°C. Avoid repeated transfers between –80°C and –20°C. SSB settles naturally over time. Before aliquoting, **ALWAYS** heat SSB to 45°C for 5 minutes and mix thoroughly to fully resuspend the protein.

Reaction Protocol

General Handling Notes

Keep all SHARP reaction components on ice during reaction assembly. Thaw all components completely on ice and mix thoroughly, as reagents can settle or separate during storage. **ALWAYS** warm SSB to 45°C and vortex thoroughly before use. Enzyme solutions are viscous and require slow, careful pipetting to ensure accurate transfer. For multiple reactions, preparation of a master mix is recommended to minimize pipetting variability and improve consistency.

Good laboratory practice is essential to prevent contamination. Perform reaction setup in a clean area or biosafety cabinet, use aerosol-resistant filter tips, and wipe work surfaces with a DNA-decontamination solution. After amplification, open tubes only in a designated post-amplification area to avoid releasing high-copy amplicons.

Standard 20 µL Reaction Composition

Component	**Volume Needed**
SHARP Reaction Buffer (10X)	2 µL
dNTP Mix (10 mM each)	1 µL
Target DNA Template[1]	Variable
Forward Primer[2]	Variable
Reverse Primer[2]	Variable
PcrA-M6 Helicase	1 µL
SSB Protein	1 µL
Bst-LF Polymerase	1 µL
ATP (100 mM)	1 µL
Nuclease-free Water	To 20 µL total volume
Optional: DNA-binding Dye	*Follow dye instructions*
Total[3]	**20 µL**

1. Lower target DNA copy numbers generally require longer amplification times.
2. A final concentration of **1 µM** for each primer is recommended as a starting point. Primer design and concentrations typically benefit from further optimization to improve amplification speed, yield, and suppression of erroneous products.
3. The SHARP reaction is fully scalable. Adjust all components proportionally. Smaller reaction volumes are not recommended due to pipetting inaccuracies, while larger volumes are acceptable if uniform, effective heating can be maintained.

Standard 20 µL Reaction Setup

The following procedure provides a recommended starting point for assembling and running a 20 µL SHARP reaction. Reaction conditions may be further optimized for specific assays or sample types.

1. Calculate Variable Volumes

Determine the volumes of all variable components (template, primers, and optional DNA-binding dye). Calculate the volume of nuclease-free water required to bring the final reaction volume to 20 µL**.**

2. Thaw and Mix Reagents

Thaw all components completely on ice. Mix thoroughly and briefly centrifuge to collect contents.

3. Combine Reaction Components

Combine all components except ATP into a reaction vessel on ice according to volumes calculated in step 1. **DO NOT** add ATP at this stage as it initiates the reaction.
Note: avoid creating bubbles when pipetting.

4. Initiate the Reaction

Add ATP on ice. Mix gently and briefly centrifuge.
Note: although the reaction begins upon ATP addition, progression is slow while the reaction remains on ice.

5. Incubate the Reaction

Incubate reactions at 65 °C for 40 minutes.
Note: Amplification time depends on assay design and can be optimized. Excessively long incubations may lead to the formation of erroneous amplification products.

6. Optional Real-Time Monitoring

If using a DNA-binding dye (e.g., EvaGreen or SYBR Green), monitor fluorescence in real time at a constant 65 °C on a compatible instrument.

Preparing Multiple Reactions

For experiments involving multiple SHARP reactions, preparation of a mastermix is recommended to reduce pipetting variability and improve consistency. A mastermix should contain all components that remain constant across the set of reactions, scaled appropriately for the number of reactions being prepared. Any components that differ between reactions should be added individually.

Post-Amplification Cleanup

SHARP amplification products should be purified prior to downstream analysis such as agarose gel electrophoresis or DNA sequencing. Purify each reaction using a standard PCR cleanup method (e.g., silica column or magnetic bead–based cleanup) according to the manufacturer's instructions. Elute the cleaned product in nuclease-free water or buffer appropriate for the intended downstream application.

Positive Control Reaction

The SHARP Positive Control Kit provides a λ-DNA template (5 ng/µL) and a premixed λ-200 primer pair (20 µM each) for verifying kit performance and confirming proper reaction setup. To run the positive control, substitute **1 µL** of the λ-DNA template and **1 µL** of the λ-200 primer mix into the 20 µL reaction setup described above. Add nuclease-free water to bring the reaction volume to **20 µL** (11 µL needed if not running with DNA dye). Incubate at 65°C for 30 minutes.

Under proper handling and recommended running conditions, the positive control reaction should exhibit the following performance characteristics:

- Amplification of a 198 bp segment of lambda phage DNA (segment 43,956–44,153)
- Detectable amplification typically observed between 8-12 minutes at 65 °C
- Completion of amplification between 15-20 minutes, identifiable through real-time fluorescence monitoring.