proACE™ Bst Fluorescent Hotstart

#GE91.1600 (1600U)
(FOR RESEARCH ONLY)

Product: Loop-mediated Isothermal Amplification is a powerful DNA amplification method that works at a constant temperature (usually 60ºC-65ºC) without the need of a thermal cycler. It achieves extremely rapid and specific amplification (typically 10^9–10^{10} copies in 15–60 minutes) using 4 to 6 specially designed primers and a strand-displacing DNA polymerase.

proACE™ Bst Fluorescent Hotstart is a kit containing all components for Loop-mediated Isothermal Amplification. It comprises proACE™ Hotstart Bst Polymerase (the large fragment of *Bacillus stearothermophilus* DNA polymerase, expressed in *E. coli*, combined with a proprietary hot start technology). This large fragment of Bst DNA Polymerase preserves 5´→3´ polymerase activity for DNA synthesis but is devoid of 5´→3´ exonuclease activity, thereby facilitating the strand displacement mechanism that is critical to Loop-mediated Isothermal Amplification. The reversible hot start technology allows for setting up the experiment at ambient temperature, reducing non-specific amplification that may occur below 45ºC, which is ideal for point-of-care and field testing. This kit is supplied with a 20x concentrated fluorescent dye for real-time detection using any qPCR thermocycler.

Applications:
Loop-mediated Isothermal Amplification
Whole Genome Amplification (WGA)
Multiple Displacement Amplification (MDA)
Development of Molecular Diagnostic Test
Point-of-care Testing and Field Testing

Contents: #GE91.1600 contains sufficient enzyme, buffers, and dye for 200 reactions of 25µl.

Properties:
Reversible hot start technology for ultra-sensitive and reliable detection
Low detection limit (as low as 3 target copies per microliter)
Fast amplification at broad temperature range (55ºC-70ºC)

Storage: Store at -20ºC and protected from light for at least 2 years. Repeated thaw/freeze cycles (up to 20 times) have no negative impact on performance.

Loop-mediated Isothermal Amplification

GRiSP recommends to use default Primer Explorer v5 settings (free software from Eiken Chemical) for designing primers with predicted melting temperature of approximately 60ºC. A 10x Primer Mix contains all 4 (or 6 primers if you include Loop primers. Loop primers are not essential but by binding to the loops formed during amplification, they speed up the reaction (often reduce time to half), and increase sensitivity) in TE Buffer at the following concentrations: 6µM of both FIP and BIP (Forward and Backward inner primers), 2µM of both F3 and B3 (Forward and Backward outer primers) 2µM, and (optional) 4-8µM of both LoopF and LoopB (Forward and Backward Loop primers).

1. Set-up

Component	Volume	Final Conc.
proACE™ Bst Fluorescent Hotstart (8U/µl)	1.0 µl	8U
proACE™ Bst Buffer A (10X)	2.5 µl	1X
proACE™ Bst Buffer B (5X)	5.0 µl	1X
proACE™ Fluorescent Dye (20X)	1.25 µl	1X
10x Primer Set (see above)	2.5 µl	1X
Template DNA	Variable	
PCR –grade water (RNase-free)	up to 25 µl	

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control (no RTase) for the detection of possible contaminants, by mixing all components (N+1), except template DNA, dividing the mixture equally into each tube and then add template DNA or PCR grade water in case of the control to the individual tubes or wells of suitable plates.

2. Protocol

1. Incubate at 65ºC for 30 minutes. If needed, temperature can be adjusted between 55ºC and 70ºC and time can be extended for low-copy or difficult targets)
2. For real-time reading, follow the reaction in a qPCR instrument, using the FAM-channel acquiring data every 10-15 seconds.
3. If required, proACE™ Hotstart Bst Polymerase can be heat-inactivated by incubation at 80ºC for 10 minutes.

Please note that it if you have no access to a device for the detection of fluorescence, we recommend #GE90.0100 proACE™ Bst Visual Mix, which allows for identifying positive and negative results visually (color shift from orange to yellow).

Strand displacement

For strand displacement amplification, it is recommended to incubate for 30-60 minutes at 65ºC and to deactivate by incubation at 80ºC for 10 min.