BIO-X-ACT™ Short DNA Polymerase

Shipping: On Dry/Blue Ice
Exp. Date: See vial
Batch No.: See vial
Concentration: 4u/µl

Catalog numbers
BIO-21064 : 250 Units: 62.5µl
BIO-21065 : 500 Units: 125µl

Store at –20°C

DATA SHEET

Storage and stability:
The BIO-X-ACT Short DNA Polymerase shipped on Dry/Blue Ice and can be stored for up to 12 months at -20°C.

Storage Buffer:
20mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1mM EDTA, 2mM DTT, 50% glycerol, and stabilizers.

Safety precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Unit Definition:
One unit is defined as the amount that incorporates 10nmoles of dNTPs into acid-precipitable form in 30 minutes at 72°C.

Associated Activities:
Endonuclease and exonuclease activities were not detectable after 4 hours of incubation of 1µg of pBR322 plasmid DNA and 0.5µg Hind III-digested Lambda DNA at 72°C in the presence of 20 units of BIO-X-ACT.

Notes:
BIO-X-ACT is a Trademark of Bioline.
This product insert is a declaration of analysis at the time of manufacture.
Research Use Only.

Features

- Amplifies fragments up to 5Kb
- Higher fidelity than standard *Taq*
- Ideal for problematic templates that fail with standard *Taq* DNA polymerases
- Reproducible results

Applications

- For high fidelity PCR
- Products suitable for cloning

Description

BIO-X-ACT™ Short DNA polymerase is a high-performance enzyme preparation specifically designed for difficult PCR applications requiring both high processivity and high fidelity that would normally fail with *Taq* DNA polymerase.

BIO-X-ACT Short DNA Polymerase is recommended for short genomic DNA fragments of up to 3Kb.

Components

	250 Units	500 Units
BIO-X-ACT Short DNA Polymerase	62.5µl	125µl
10x OptiBuffer	1.2ml	2 x 1.2ml
50mM $MgCl_2$ Solution	1.2ml	1.2ml
5x Hi-Spec Additive	1.2ml	1.2ml

Reagent Specifications:
5x Hi-Spec Additive is a specificity enhancer. If necessary, re-dissolve Hi-Spec by heating to 70°C and vortexing.

PCR Protocols

Recommended parameters for PCR of 1Kb fragment with BIO-X-ACT Short DNA Polymerase

Components	Volume
10x OptiBuffer	5µl
50mM $MgCl_2$ Solution	2µl
100mM dNTP	1µl
Hi-Spec Additive (if require)	2.5µl
Template Lambda DNA 5ng/µl	10µl
Primer mix 100µM	1µl
BIO-X-ACT Short 4u/µl	1µl
Water (ddH_20)	Up to 50µl

Reaction Mix:

Cycling Parameters	Stage of incubation	Incubation Temperature	Incubation Time
1x	Initial denaturation	95°C	5 min
30x	Denaturation	94°C	30 sec
	Annealing	55°C*	30 sec
	Extension	72°C	1 min
1x	Final Elongation	72°C	10 min

* Annealing temperature is primer-dependent

This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

General Considerations:

- **Difficult Templates:** BIO-X-ACT provides high performance and specificity, even with 'dirty' DNA or difficult templates with an unfavorable nucleotide composition. In contrast to other standard 3'-5' proofreading polymerases, BIO-X-ACT can be used in combination with degenerate or imperfect matching primers.
- **Cycling Conditions:** The annealing temperature should be approximately 5°C lower than the predicted Tm. The extension temperature is usually between 68-72°C. Allow 1 min/kb.

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- High Fidelity: BIO-X-ACT Short possesses higher fidelity than *Taq* DNA polymerase.

- Higher Specificity: BIO-X-ACT is supplied with a vial of a very efficient specificity enhancer. 5x Hi-Spec Additive helps to prevent the formation of false background bands and smearing, especially on difficult templates. Hi-Spec Additive should be used at 1.0-2.0x final concentration - the optimal amount required should be determined for each individual experiment.

PCR Troubleshooting Guide

Observation	Possible cause	Recommended solution(s)
No or low PCR yield	GC-rich template	Use Hi-Spec Additive
	Enzyme concentration too low	Increase the amount of enzyme in 0.5U increments
	Magnesium concentration too low	Increase concentration in 0.25mM increments
	Primer concentration not optimized	Titrate primer concentration (0.3-1µM); ensuring that both primers are equimolar
Multiple bands	Primer annealing temperature too low	Increase annealing temperature. Primer annealing should be at least 5^{o}C below the calculated Tm of primers.
	Master mix left at room temperature	Prepare and keep master mixes on ice
	Low specificity	Try Hi-Spec Additive
Smearing or artefacts	Template concentration too high	Prepare serial dilutions of template
	Too Many cycles	Reduce the cycle number by 3-5 to remove non-specific bands
	Enzyme concentration too high	Decrease the amount of enzyme in 0.5U increments
	Extension time too long	Reduce extension time in 0.5-1 minute increments

Product Citations:

1. Grosse, C., *et al. Appl. Environ. Microbiol.* **74(15)**, 4923–4933 (2008).
2. Oliver, M.K. and Piertney, S.B. *Immunogene.* **58**, 390–395 (2006).
3. Donato, G.M., *et al. J. Bacteriol.* **187(22)**, 7579-7588 (2005).
4. Gow, J.L., *et al. Genetica* **124(1)**, 77-83 (2005).

Associated Products:

Product Name	Pack Size	Cat. No
dNTP Set	4 x 25µmol	BIO-39025
dNTP Mix 100mM total	1 x 500µl	BIO-39028
4x PolyMate Additive	2 x 1.2ml	BIO-37041
SureClean Plus	1 x 5ml	BIO-37047
Agarose	100g	BIO-41026