



## BIO-X-ACT™ Long DNA Polymerase

Shipping: On Dry/Blue Ice Catalog numbers

Exp. Date: See vial BIO-21049 : 250 Units: 62.5µl

Batch No.: See vial BIO-21050 : 500 Units: 125µl

**Store at -20°C**

### **Storage and stability:**

The BIO-X-ACT Long 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 10nmol 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 20Kb
- Higher fidelity than standard Taq
- Ideal for problematic templates that fail with standard *Taq* DNA polymerases
- Reproducible results

### **Applications**

- For high fidelity PCR of long DNA fragments

- Products suitable for cloning

### Description

BIO-X-ACT™ Long DNA Polymerase is specifically designed for difficult/problematic PCR applications requiring high processivity with fidelity that would normally fail with standard *Taq* polymerases. The polymerase has been optimized for a wide variety of templates and an additional 50mM of MgCl<sub>2</sub> solution is included should any fine adjustments be required. BIO-X-ACT Long DNA Polymerase is recommended for long Genomic DNA fragments of between 3-20Kb.

### Components

	250 Units	500 Units
BIO-X-ACT Long DNA Polymerase	62.5µl	125µl
10x OptiBuffer	1.2ml	2 x 1.2ml
50mM MgCl <sub>2</sub> 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.

### Reaction Mix:

Cycling Parameters	Stage of incubation	Incubation Temperature	Incubation Time
1x	Initial denaturation	94°C	2 min
	Annealing	*	1 min
30x	Denaturation	94°C	30s
	Annealing	*	30s
	Extension	68°C	20 min
1x	Final Elongation	68°C	20 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**

## PCR Protocols

Recommended parameters for PCR of 20Kb fragment with BIO-X-ACT Long DNA Polymerase

Components	Volume
10x OptiBuffer	5µl
50mM MgCl <sub>2</sub> Solution	1µl
100mM dNTP	0.5µl
DMSO (recommended)*	2.5µl
Template Lambda DNA 5ng/µl	1µl
Primer mix 100µM	0.3µl
BIO-X-ACT Long 4u/µl	1µl
Water (ddH <sub>2</sub> O)	Up to 50µl

\* The use of DMSO is strongly recommended. If using Hi-Spec Additive, add 10-20µl to get 1.0- 2.0x final concentration. Adjust the volume of water accordingly. It is not recommended to use both DMSO and Hi-Spec Additive together.

### General Considerations:

**Long-Region Applications:** Optimal composition of buffer and polymerase enables BIO-X-ACT Long to span the primer extension over long regions and demonstrate high processivity by reducing premature strand termination and template degradation. Using long primers at elevated Mg<sup>2+</sup> concentrations, >30Kb or 20Kb products can be achieved from lambda templates or genomic DNA, respectively.

**Extension Times:** The extension time depends on the amplicon length and complexity. The longer and more complex the amplicon, the longer the extension time required. We recommend using 1Kb/ minute.

**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 imperfectly matching primers.

**Cloning:** BIO-X-ACT™ is recommended for direct gene cloning.

**High Fidelity:** BIO-X-ACT possesses provides higher fidelity than *Taq* and in contrast with other proofreading enzymes, BIO-X-ACT does not degrade primers.

**DMSO:** We strongly recommend the use of 5% DMSO (supplied) in the reaction mix to increase the specificity, sensitivity and yield of BIOX- ACT when performing long range PCR.

**Hi-Spec Additive:** Alternatively, 5x Hi-Spec Additive (supplied) can also be used. Hi-Spec Additive helps to prevent the formation of false background bands and smearing, especially on difficult templates. Please note that DMSO and Hi-Spec Additive should not be used together.

## PCR Troubleshooting Guide

Observation	Possible cause	Recommended solution(s)
No or low PCR yield	Difficult templates (AT and GC-rich)	Add DMSO or Hi-Spec Additive to lower the melting profile and improve performance
	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 have the same concentration
	Template concentration too low	Increase template concentration or add DMSO
Multiple bands	Primer annealing temperature too low	Increase annealing temperature. Primer annealing should be at least 5°C below the calculated T <sub>m</sub> of primers.
	Master mix left at room temperature	Prepare and keep master mixes on ice
	Low specificity	Add DMSO or 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. Mullane, N., *et al. Appl. Environ. Microbiol.* **74(12)**, 3783–3794 (2008).
2. Miller, H. C., *et al. J. Hered.* **98(7)**, 666-677 (2007).
3. Eliahu, N., *et al. Eukaryotic Cell* **6(3)**, 421-429 (2007).
4. Shirak, A., *et al. Gene.* **174(3)**, 1573-1581 (2006).
5. Meissner, C., *et al. Expt. Gerontol.* **41(4)**, 518-524 (2006).
6. Wright, J.C., *et al. J. Bacteriol.* **186(20)**, 6970-6982 (2004).

### Associated Products:

	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