ACCUZYME™ DNA Polymerase

Shipping: On Dry/Blue Ice
Batch No.: See vial
Concentration: 2.5u/µl

Catalog numbers
BIO-21051 : 250 units (100µl)
BIO-21052 : 500 units (200µl)

Store at –20°C

BIOLINE
A Meridian Life Science® Company

Storage and stability:
The ACCUZYME is shipped on dry/blue ice. On arrival store at -20°C for optimum stability. Repeated freeze/thaw cycles should be avoided.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:
Please refer to the material safety data sheet for further information.

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

Quality control specifications:
Bioline operates under ISO 9001 Management System. ACCUZYME and its components are extensively tested for activity, processivity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination prior to release.

Notes:
Research use only.

Features

- Very high yield
- High fidelity
- Amplifies fragments up to 5kb

Applications

- Ideal for ultra-high fidelity for subsequent cloning
- Blunt-end cloning
- Site-directed mutagenesis

Description

ACCUZYME™ DNA polymerase is a thermostable enzyme possessing 5'-3' DNA polymerase and 3'-5' proofreading exonuclease activities, offering high fidelity, even with demanding applications. ACCUZYME produces blunt-ended amplicons of up to 5Kb in length. ACCUZYME is supplied with 10x Reaction Buffer containing Mg^{2+}, which provides optimal final reaction conditions for most experiments. In order to allow optimization of reaction conditions, additional $MgCl_2$ is provided.

Components

	250 Units	500 Units
ACCUZYME DNA Polymerase	100µl	200µl
10x AccuBuffer	1.2ml	2 x 1.2ml
50mM $MgCl_2$ Solution	1.2ml	1.2ml

PCR Reaction Conditions (for a 50µl reaction)

The following protocol is for a standard 50µl reaction and can be used as a starting point for reaction optimization. Please refer to the Important Considerations and PCR Optimization section.

10x AccuBuffer	5µl
50mM $MgCl_2$ Solution	Optional
100mM dNTP Mix (see below)	0.5 - 1µl
Template	as required
Primers 20µM each	1µl
ACCUZYME DNA Polymerase 2.5U/µl	1 - 3µl
Water (ddH_2O)	up to 50µl

PCR cycling conditions:

Step	Temp.	Time	Cycles
Initial Denaturation	95°- 98°C	3 min	1
Denaturation	95°- 98°C	15s	25-35
Annealing*	55°-60°C	15s	
Extension	72°C	1.5 - 2 min/kb	

*Annealing temperature is primer dependent.

The conditions above are intended as a guide only; conditions will vary from reaction to reaction and may need optimization.

Important considerations and PCR optimization

The optimal conditions will vary from reaction to reaction and are dependent on the template/primers used.

10x AccuBuffer: The 10x AccuBuffer comprises of 600mM Tris-HCl, 60mM $(NH_4)_2SO_4$, 100mM KCl, 20mM $MgSO_4$, pH 8.3 at 25°C.

The Mg^{2+} concentration in the 1x AccuBuffer is 2mM, this is the optimum concentration for ACCUZYME for most PCR reactions and should only be adjusted if necessary.

ACCUZYME DNA Polymerase: We recommend starting with 1µl (2.5 Units) of ACCUZYME in a 50µl reaction.

Primers: Forward and reverse primers are generally used at the final concentration of 0.2-0.6µM each. As a starting point, we recommend using 0.4µM final concentration (*i.e.* 20pmol of each primer per 50µl reaction volume). Too high a primer concentration can reduce the specificity of priming, resulting in non-specific products.

When designing primers we recommend using primer-design software such as Primer3 (http://frodo.wi.mit.edu/primer3) or visual OMP™ (http://dnasoftware.com). Primers should have a melting temperature (Tm) of approximately 60°C.

Template: The amount of template in the reaction depends mainly on the type of DNA used. For templates with low structural complexity, such as plasmid DNA, we recommend using 50pg-10ng DNA per 50µl reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200ng DNA per 50µl reaction, this can be varied between 5ng-500ng. It is important to avoid using template re-suspended in EDTA-containing solutions (*e.g.* TE buffer) since EDTA chelates free Mg^{2+}.

Website: www.bioline.com/ email: info@bioline.com

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No PCR product	Missing component	- Check reaction set-up and volumes used
	Defective component	- Check the aspect and the concentrations of all components as well as the storage conditions. If necessary test each component individually in controlled reactions
	Enzyme concentration too low	- Increase enzyme quantity in 0.5U (0.2µl) increments
	Cycling conditions not optimal	- Decrease the annealing temperature - Run a temperature gradient to determine the optimal annealing temperature - Increase the extension time, especially if amplifying a long target - Increase the number of cycles
	Difficult template e.g. GC or AT-rich, or high level of secondary structure	- Increase initial denaturation time to 5 minutes - Increase denaturation time
Smearing or Non-Specific products	Excessive cycling	- Decrease the number of cycles
	Extension time too long	- Decrease the extension time
	Annealing temperature too low	- Increase the annealing temperature
	Primer concentration too high	- Decrease primer concentration
	Contamination	- Replace each component in order to find the possible source of contamination - Set-up the PCR reaction and analyze the PCR product in separated areas

Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact your local distributor or our Technical Support with details of reaction setup, cycling conditions and relevant data.

Email: tech@bioline.com

Associated Products

Product Name	Pack Size	Cat. No.
dNTP Set	4 x 25µmol	BIO-39025
dNTP Mix	500µl	BIO-39028
ACCUZYME™ Mix	2 x 1.25ml	BIO-25027

Product Citations

1. Kitazono, A.A. *Gene* doi:10.1016/j.gene.2011.06.006 (2011).
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5. Cheng, C., *et al. Mol. Cell. Biol.* **31**, 983-997 (2011).
6. Chakrabarti, M., *et al. Virol. J.* 7, **181** (2010).
7. Silvestrini, F., *et al. Mol.Cell. Prot.,* **9**, 1437-48 (2010).
8. Williamson, D. S., *et al. Appl. Microbiol. Biotechnol.* **88**, 143-153 (2010).
9. Johnson M., *et al. NAR* **37(14)**, e98 (2009).
10. Pacheco, A., *et al. Microbiol.* **155**, 2021-2028 (2009).

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