ACCUZYME™ Mix

Shipping: On Dry/Blue Ice

Batch No.: See vial

Concentration: 2x

Catalog numbers

BIO-25028: 500 x 50 µL reactions (10 x 1.25 mL)

Store at –20 °C

Storage and stability:
The ACCUZYME Mix 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.

Quality control specifications:
ACCUZYME Mix 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:
ACCUZYME is a trademark of Bioline Reagents Ltd.
Research use only.

Features

- High fidelity coupled with high yield
- Amplifies fragments up to 5 kb
- Convenient pre-mixed, pre-optimized 2x solution
- Reduced risk of contamination
- Dramatically decreases the time required for reaction set-up
- Reproducible results

Applications

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

Description

ACCUZYME™ Mix is a convenient ready-to-go 2x reaction mix designed to maximize experiment reproducibility. ACCUZYME Mix contains ACCUZYME DNA Polymerase, $MgCl_2$ and ultra-pure dNTPs manufactured by Bioline. The mix is optimized and ready-to-use, the user is simply required to add water, template and primers.

ACCUZYME Mix dramatically reduces the time needed to set up reactions, thereby minimizing the risk of contamination. Greater reproducibility is ensured, by a reduction in the number of pipetting steps that can lead to pipetting errors. ACCUZYME Mix is supplied with an additional 50 mM $MgCl_2$ solution for optional optimization of reaction conditions.

Components

	500 Reactions
ACCUZYME™ Mix, 2x	10 x 1.25 mL
50 mM $MgCl_2$ Solution	1.2 mL

Standard ACCUZYME Mix Protocol

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

PCR reaction set-up:

ACCUZYME™ Mix, 2x	25 µL
Primers 20 mM each	1 µL
Template	as required
Water (ddH_2O)	up to 50 µL

PCR cycling conditions:

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

*Annealing temperature is primer dependent

The conditions above are intended for use 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.

Mg^{2+} concentration: The Mg^{2+} concentration in the 2x mix is 4 mM (2 mM final concentration), this is the optimum concentration for ACCUZYME Mix for most PCR reactions and should only be adjusted if necessary.

Primers: Forward and reverse primers are generally used at the final concentration of 0.2-0.6 µM each. As a starting point, we recommend using 0.4 µM final concentration (*i.e.* 20 pmol of each primer per 50 µL 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 50 pg-10 ng DNA per 50 µL reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200 ng DNA per 50 µL reaction, this can be varied between 5 ng-500 ng. 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@meridianlifescience.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
	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

Product Citations

ACCUZYME DNA Polymerase

1. Kitazono, A.A., *Gene* doi:10.1016/j.gene.2011.06.006 (2011).
2. Batchelor, D.J., *et al. Am. J. Physiol.* **300,** R67-R75 (2011).
3. Chiang, C., *et al. J. Bacteriol.* **193,** 52-62 (2011).
4. Chin, G.L., *et al. Appl. Envir. Microbiol*. **77**, 3451-3460 (2011).
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).
11. Wilson, A. C., *et al. J. Bacteriol.* **190(15)**, 5522-5525 (2008).

ACCUZYME MIX

1. Padmashali R.M. & Andreadis. S.T., *Biomaterials* **32 (12),** 3330-3339 (2011).
2. Potula, S. K., *et al. Transgen. Res.* **17(1)**, 19-32 (2008).
3. Jury, F., *et al. Med. Microbiol.* **55**, 1053-1060 (2006).

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@meridianlifescience.com

Associated Products

Product Name	Pack Size	Cat. No.
dNTP Set	4 x 25 µmol	BIO-39025
dNTP Mix	500 µL	BIO-39028
ACCUZYME™ DNA Polymerase	250 units (100 µL)	BIO-25021

Bioline USA Inc.
USA

Tel: +1 901 382 8716
Fax: +1 901 382 0027

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