EPIK™ Amplification Kit

Shipping: On Dry/Blue ice

Catalog numbers:

BIO-66025: 200 x 50µl reactions: 4 x 1.25ml

Batch No.: See vial

BIO-66026: 500 x 50µl reactions: 10 x 1.25ml

Concentration: 2x

Store at –20°C

BIOLINE

A Meridian Life Science® Company

Storage and stability:
EPIK™ Amplification Kit 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:
Bioline operates under ISO 9001 Management System. EPIK Amplification Mix and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination prior to release.

Notes:
Research use only.

Description

EPIK™ Amplification Kit has been developed to overcome the challenges associated with the bisulfite-modified DNA templates, to deliver highly reproducible and sensitive PCR results, even with long amplicons and low concentrations of template DNA. EPIK Amplification Kit contains EPIKORE™ buffer, the latest advances in buffer chemistry and enhancers, together with MyTaq™ HS, an antibody-mediated hot-start DNA polymerase system, make the EPIK Amplification Kit highly suited for high-throughput epigenetic assays and bisulfite sequencing reactions.

Components

	200 Reactions	500 Reactions
EPIK Amplification Mix , 2x	4 x 1.25ml	10 x 1.25ml

Standard Protocol

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

PCR set-up:

Bisulfite-treated DNA	2-200ng
Primers (25µM each)	1µl
2x EPIK Amplification mix	25µl
Water (dH_2O)	Up to 50µl

Step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1
Denaturation	95°C	15s	30-40
Annealing*	User determined	15s	
Extension*	72°C	30s	

Important Considerations and PCR Optimization

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

- **Template:** The amount of template in the reaction depends mainly on the source of DNA as well as the conversion kit used. Higher amounts of template (up to 200ng per reaction) are required for DNA with high structural complexity such as that from eukaryotes. In addition, when the template has been treated with conversion kits with a greater tendency to fragment the DNA, a higher amount of template is required, especially if the desired amplicon length exceeds 500bp
- The converted DNA has similar optical properties to single-stranded RNA because it has both high uracil content and is mostly made up of non-complementary nucleic acid which can be considered as single-stranded. Quantification should be performed with methods adapted to single stranded nucleic acid analysis (e.g. Nanodrop with RNA settings). We suggest using a value of 40µg/ml^{-1} for an OD_{260} of 1.0 when determining the concentration of converted DNA
- **PCR controls:** We recommend running a no-conversion control where the converted template is substituted by the non-converted DNA. This will check for the specificity of the primers used
- **Primers:** When designing primers, it is important to consider the effect that bisulfite treatment has on DNA. Bisulfite treated DNA is no longer complementary double stranded DNA so the primer set has to be specifically designed for the forward or reverse strand. Consequently, we recommend using specific online primer-design tools such as "Methprimer" (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi) or "BiSearch" (http://bisearch.enzim.hu/?m=search). Primers should have a melting temperature (Tm) of approximately 60°C
- We recommend a final primer concentration of 0.4µM (Forward and reverse primers are generally used at the final concentration of 0.2-0.6 µM each *i.e.* 20pmol of each primer per 50µl reaction volume). If necessary, the primer concentration can be optimized by titration. Too high a primer concentration can reduce the specificity of priming, resulting in non-specific products
- Amplicons of up to 500bp in length can be generated with ease. However, amplicon length should not exceed 1500bp due to the fragmented nature of bisulfite-treated DNA template. The extent of DNA fragmentation is dependent upon which bisulfite-conversion procedure is used
- **Annealing temperature and time:** The optimal annealing temperature is dependent upon the primer sequences and is usually 2-5°C below the lower Tm of the primer pair. We recommend starting with a 55°C annealing temperature and, if necessary, running a temperature gradient to determine the optimal annealing temperature
- **Extension temperature and time:** The extension step should be performed at 72°C. The extension time depends on the length of the amplicon and the complexity of the template. An extension time of 30s is sufficient for amplicons under 500bp. For amplification of fragments from 500bp up to 1.5kb use an extension step of 60s.
- **Cycle number:** We recommend starting with 35 cycles and, if necessary, optimizing this parameter. An excess of cycles may generate diffuse bands, too few may result in weak bands or no amplification

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No PCR product	Missing component	- Check reaction set-up and volumes used
	Defective component	- Check for precipitate in solution/reagent 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
	Highly fragmented template	- Increase the amount of template - Increase number of cycles - Use another conversion kit - Design new amplicon with smaller size
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 - Run a temperature gradient to determine the optimal 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 and analyze the PCR product in separate areas - Non-specific primers for bisulfite treated-DNA reaction

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 set-up, cycling conditions and relevant information.

Email: tech@bioline.com

Associated Products

Product Name	Pack Size	Cat No
EPIK Fast Quantification Hi-ROX Kit	200 Reactions	BIO-66029
EPIK Fast Quantification Lo-ROX Kit	200 Reactions	BIO-66027
Agarose	500g	BIO-41025
Agarose Tablets	300g	BIO-41027
HyperLadder™ 1kb	200 Lanes	BIO-33025
SureClean Plus	1 x 5ml	BIO-37047

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