RANGER Mix

Shipping: On Dry/Blue Ice

Batch No.: See vial

Concentration: 2x

Catalog numbers

BIO-25051: 100 x 50µl reactions: 2 x 1.25ml

BIO-25052: 500 x 50µl reactions: 10 x 1.25ml

Store at –20°C

BIOLINE

A Meridian Life Science® Company

Storage and stability:
The RANGER 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:
RANGER 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

RANGER Mix is a newly developed ready-to-use high-performance 2x mix, specifically designed to amplify long genomic DNA templates of 10kb or greater with extreme sensitivity. Owing to its antibody-based hot-start property, RANGER Mix has the advantage of avoiding unwanted non-specific amplification such as primer-dimer formation. This hot-start enzyme preparation from Bioline is supplied as a 2x formulation containing RANGER DNA Polymerase, dNTPs, $MgCl_2$ and enhancers at optimal concentrations, removing the need for optimization and delivering superior amplification.

Components

	100 Reactions	500 Reactions
RANGER Mix, 2x	2x 1.25ml	10 x 1.25ml

Standard RANGER Mix Protocol

The following protocol is for a standard 50µl amplification of a 10kb fragment 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:

Template	As required
Primers (20µM each)	1µl
RANGER Mix, 2x	25µl
Water (ddH_2O)	up to 50µl

PCR cycling conditions:

Step	Temperature	Time	Cycles
Initial denaturation	95°C	1min	1
Denaturation	98°C	10s	30
Annealing/Extension	*°C	8min**	

* Temperature is primer dependent

** For 10kb amplicons. For longer amplification please refer to Important Considerations and PCR Options section.

This data is intended as a guide only; conditions will vary depending on the primer/template system 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.

Buffer: The 2x RANGER Mix contains dNTPs, $MgCl_2$ (1.5mM final concentration) stabilizers and enhancers. The concentration and ratio of each component has been extensively optimized, reducing the need for further optimization. Addition of $MgCl_2$ and PCR enhancers such as DMSO, etc. are not necessary.

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 a 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) with monovalent and divalent cation concentrations of 45mM and 1.5mM respectively. 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+}.Repeated freeze/thawing of the template is not recommended, especially when amplifying long fragments of DNA.

Initial Denaturation: The initial denaturation step is required to activate the enzyme and fully melt the template. For most PCR 1 minute at 95°C is sufficient to melt the DNA template, however we recommend up to 3min for complex templates such as eukaryotic genomic DNA.

Denaturation: We recommend a 10s cycling denaturation step at 98°C. Increasing this step up to 20s may improve problematic DNA.

Annealing/Extension: The optimal annealing temperature for this step is dependent upon the primer sequences and is usually 2-5°C below the lower Tm of the pair. We recommend running a temperature gradient to determine the optimal annealing/extension temperature.

The allocated time for the annealing/extension step depends on the length of the amplicon and the complexity of the template, the more complex the amplicon, the longer the extension time. We recommend extension time of 45s/kb up to 60s/kb.

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No PCR product	Missing component	- Check reaction set-up
	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 - Redesign primers
	Cycling conditions not optimal	- Run a temperature gradient to determine the optimal annealing/extension temperature - Increase the extension time, especially if amplifying a long target - Increase the number of cycles
	Difficult template	- Increase the initial denaturation time up to 3min
Smearing or Non-Specific products	Excessive cycling	- Decrease the number of cycles
	Annealing/extension time too long	- Decrease the annealing/extension time
	Annealing/extension temperature too low	- Increase the annealing/extension temperature
	Primer concentration too high	- Decrease primer concentration
	Suboptimal primer design	- Check that the primers are working in a control reaction - Check primer design
	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
Low Yield	Insufficient cycling	- Increase the number of cycles
	Annealing/extension time too short	- Increase the annealing/extension time up to 30s/kb
	Not enough template	- Increase template concentration

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
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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