RANGER DNA Polymerase

Shipping: On Dry/Blue Ice

Concentration: 4 U/μL

Batch No.: See vial

Catalog numbers

BIO-21121 : 250 Units

BIO-21122 : 500 Units

Store at –20 °C

bioline
meridian BIOSCIENCE

Storage and stability:
RANGER 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 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72 °C.

Quality control specifications:
RANGER 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 DNA Polymerase is a newly developed high-performance enzyme, specifically designed to amplify long genomic DNA templates of 10 kb or greater with extreme sensitivity. Owing to its antibody-based hot-start property, RANGER DNA Polymerase reactions can be setup at room temperature and have the added advantage of avoiding unwanted non-specific amplification such as primer-dimer formation. This new hot-start enzyme preparation from Bioline is supplied with 5x RANGER Reaction Buffer, a proprietary formulation containing dNTPs, $MgCl_2$ and enhancers at optimal concentrations, removing the need for optimization and delivering superior amplification.

RANGER DNA Polymerase possesses higher fidelity than *Taq* polymerase and together with the novel RANGER Buffer, provides accurate long-range amplification of standard and complex templates.

Components

	250 Units	500 Units	2500 Units
RANGER DNA Polymerase	1 x 62.5 μL	1 x 125 μL	2 x 312.5 μL
5x RANGER Reaction Buffer	1 x 630 μL	1 x 1.2 mL	2 x 1.2 mL

Standard RANGER Protocol

The following protocol is for a standard 50 μL amplification of 10 kb fragments 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:

5x RANGER Reaction Buffer	10 μL
Template	as required
Primers 20 μM each	1 μL
RANGER DNA Polymerase	1 μL
Water (dH_2O)	up to 50 μL

PCR cycling conditions:

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

* Temperature is primer dependent

** For 10 kb 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.

5x RANGER Reaction Buffer: The 5x RANGER Reaction Buffer contains dNTPs, $MgCl_2$ (1.5 mM final concentration) and enhancers. The concentration and ratio of each component have been extensively optimized, reducing the need for further optimization. Additional $MgCl_2$ PCR enhancers such as DMSO etc. are not recommended.

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 a 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) with monovalent and divalent cation concentrations of 45 mM and 1.5 mM 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 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+}. 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 3 min for complex templates such as eukaryotic genomic DNA.

Denaturation: We recommend a 10 s cycling denaturation step at 98 °C. Increasing this step to 20 s 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 annealing/extension time of 45 s/kb up to 60 s/kb.

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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 3 min
Smearing or Non-Specific products	Excessive cycling	- Decrease the number of cycles
	DNA polymerase concentration too high	- Decrease amount of DNA polymerase per reaction
	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 60 s/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@meridianlifescience.com

Associated Products

Product Name	Pack Size	Cat No
Agarose	500 g	BIO-41025
Agarose tablets	300 g	BIO-41027
HyperLadder™ 1 kb	200 Lanes	BIO-33025
SureClean Plus	1 x 5 mL	BIO-37047

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