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PCR amplification kit with Fusion DNA polymerase

Cat. No. KH041-100, KH041-500

Description

A set of reagents for performing PCR with high-fidelity DNA polymerase. The kit contains individual components such as magnesium ions, a mixture of deoxynucleotide triphosphates (dNTPs) and dimethyl sulfoxide, which allows you to optimize the amplification conditions for the experimenter's tasks.

Kit contents

1. Recombinant Fusion DNA polymerase, 2 U/µl (fused thermostable DNA polymerase of *P. furiosus* (Pfu) and DNA-binding protein of *S. solfataricus* (Sso7d)). Storage buffer: 20 mM Tris-HCl (pH 7.5 at 25°C), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml BSA, 0.1% Tween 20, 0.1% Triton X-100, 50% glycerol.
2. 5x reaction buffer, buffer composition: 250 mM Tris-HCl (pH 9.0 at 25°C), 175 mM KCl, 50 mM $(NH_4)_2SO_4$, 500 mM trehalose, 500 µg/ml BSA, 0.5% Tween 20, 5% glycerol.
3. 50x dNTP mixture (10 mM each).
4. 100 mM $MgCl_2$ solution.
5. 100% dimethyl sulfoxide (DMSO).
6. Water treated with diethyl pyrocarbonate, free from nucleases.

Cat.No.	Fusion DNA polymerase	5x reaction buffer	50x dNTP mixture	100 mM $MgCl_2$ solution	DMSO	Water
KH041-100	1x50 µl (100 U*)	1x0,6 ml	1x50 µl	1x0,2 ml	1x0,2 ml	1x2,0 ml
KH041-500	1x250 µl (500 U*)	2x1,5 ml	1x250 µl	1x1,0 ml	1x1,0 ml	5x2,0 ml

* One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.

Applications

Fusion DNA Polymerase has increased fidelity and speed and produces blunt-ended amplicons, making the kit a good choice for routine gene cloning and can be used to generate long or difficult amplicons by PCR.

Protocol for standard PCR reaction

1. Defrost the kit components and mix the solutions.

2. Mix individual components in a tube according to the table (for optimal results keep the components and reaction mixture on ice):

Component	Reaction mixture 25 µl	Reaction mixture 50 µl	Final concentration
5x Reaction Buffer	5 µl	10 µl	1×
50x dNTP mix (10 mM each)	0,5 µl	1 µl	200 µM of each dNTP
100 mM $MgCl_2$ solution	variable		2-5 mM [1]
DMSO (100%)	variable		up to 10% (V/V)[2]
Forward primer	variable		100-500 mM
Reverse primer	variable		100-500 mM
Template DNA	variable		1 pg - 250 ng
Fusion DNA polymerase, 2 U/µl	0,5 µl	1 µl	2.0 U/50 µl reaction mixtures
Nuclease-free water	up to 25 µl	up to 50 µl	-

[1] In most cases, we recommend using a magnesium ion concentration in the 3-4 mM range.

[2] Dimethyl sulfoxide is recommended to be added when GC-rich template DNA are used, in most cases an addition of up to 5% is sufficient, but sometimes optimization is required. It should be remembered that the addition of DMSO reduces the melting temperature of primers; 5% reduces it by approximately 2.5°C.

3. Gently mix the contents of the tube and remove droplets by short centrifugation.

Note: if using a thermal cycler without a heated lid, add a drop (25-35 µl) of mineral oil to each tube.

4. Transfer the tubes with the reaction mixture to a preheated cycler (95-98°C).

5. Use the following program for standard PCR:

Step	Temperature and time	The number of cycles
Initial denaturation	96-98°C, 30-120 seconds	1 cycle
Denaturation	96°C, 5-10 seconds	25-35 cycles
Primer annealing *	50-72°C, 10-30 seconds	
Elongation	72°C, 15-30 seconds /1 kb	
Final extension	5-10 minutes	1 cycle

* When amplifying large fragments (≥ 5 kb), it is recommended to skip this step.

6. Analyze PCR products in agarose gel. Samples must first be mixed with gel loading buffer (for example D-3002).

Storage and transportation conditions

Store at -20°C. Transportation at temperatures not exceeding +8°C is allowed for up to three days.