sbs 赛百盛

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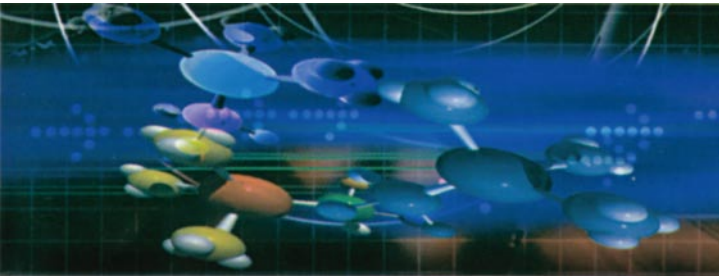
PCR-Related Products User's Instruction



SBS Genetech Co.,Ltd.

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U-Taq DNA Polymerase

EUT-500 500U

Description

U-Taq DNA Polymerase is a thermostable enzyme that can withstand prolonged incubation at temperatures up to 95℃ without significant loss of activity. The enzyme consists of a single polypeptide with a molecular weight of 94 kDa. It has a 5'->3' DNA polymerase activity and lacks a 3'->5' exonuclease activity. Its extension rate is 2~4 kb/min under standard condition, which is the highest among all thermostable DNA enzymes. The optimal reaction temperature is 70~75℃, the optimal working concentration of dNTPs is 100~300 μM,the optimal working concentration of Mg^{2+} is 2~3 mM, and the suitable pH is 8.1~9.1. The enzyme generates PCR products with 3'-dA overhangs that are suitable for TA Cloning. The amount of enzyme is 1~1.5 unit for 20 μl PCR reaction and 2~3 unit for 50 μl PCR reaction. 6 kb Lambda DNA and 2.1 kb Human genomic DNA can be amplified very well at our laboratory.

Contents

	500 units
1. U-Taq DNA Polymerase (5 units/μl)	100 μl
2. 10 x U-Taq reaction buffer	1.5 ml
3. PCR dye	1.5 ml

U-Taq DNA Polymerase Storage Buffer: U-Taq DNA Polymerase is supplied in 50 mM Tris-HCl (pH8.0), 100 mM NaCl, 0.1 mM EDTA,0.5

mM DTT, 1% TritonX-100, and 50% Glycerol.

10 x U-Taq reaction buffer: 500 mM KCl, 100 mM Tris-HCl (pH8.0), 20 mM $MgCl_2$.

Storage Conditions

Store the components at -20˚C. They are stable for more than one year under suggested storage condition. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Protocol

Note: PCR dye is an inert red dye, which greatly simplifies the operation procedure. The PCR products can be directly loaded to the agarose gel for electrophoresis with no need of additional loading buffer or tracking dye.

The following is an example of PCR reaction, only for reference.

1. In a 0.2 ml or 0.5 ml thin wall tube, add the following components:

	20 µl reaction	50 µl reaction
10 x U-Taq reaction buffer	2 µl	5 µl
Forward primer	10-20 pmol	20-50 pmol
Reverse primer	10-20 pmol	20-50 pmol
Template DNA	5-50 ng	10-100 ng
dNTPs (10 mM each)	0.5 µl	1 µl
PCR dye (optional)	2 µl	5 µl
U-Taq DNA Polymerase	1-1.5 unit	2-3 units
ddH_2O	X µl	X µl
Total	20 µl	50 µl

2. Mix gently by pipetting, close the lid and centrifuge for a few seconds.
3. Add mineral oil to each tube (this step is unnecessary when using a thermal cycler with top heating).
4. Perform PCR cycles according to the PCR condition. An example is given as follows:
   (Annealing temperature and time need to be optimized for each primer/ template combination.)

94˚C	2.5 minutes	
94˚C	45 seconds	25-35 cycles
50-65˚C	1 minute	
72˚C	1-3 minutes	
72˚C	5-10 minutes	

5. Run 2~5 μl PCR products on 1% agarose gel Stained with GoodView™ or EB.

Taq-red DNA Polymerase

ER-500 500U

Description

Taq-red DNA Polymerase is a renovated product of Taq DNA polymerase with an inert red dye, which has the same function and protocol as the ordinary Taq DNA Polymerase. Several advantages are as below:

1. The red dye enables a direct visualization of the enzyme that makes it easier to operate and observe.
2. The PCR products can be directly applied to gel electrophoresis with no additional procedure of adding loading buffer or tracking dye. As a tracking signal, the red dye has a migration rate between bromophenol blue and xylene cyanole, equivalent to the rate of 400~500bp fragment.
3. The concentration of Taq-red is exactly 1 unit/ul, hence it's easy to calculate and to maintain the accuracy and consistency between tubes.
4. The PCR products contain 3'-dA overhangs, which are suitable for TA cloning.
5. 6 kb Lambda DNA and 2.1 kb Human genomic DNA can be amplified very well at our laboratory.

Contents

	500 units
1. Taq-red DNA Polymerase (1 unit/μl)	500 μl
2. 10 x Taq-red reaction buffer	1.5 ml

Taq-red DNA Polymerase Storage Buffer: Taq DNA Polymerase is supplied in 50 mM Tris-HCl (pH8.0), 100 mM NaCl,0.1 mM EDTA, 0.5 mM DTT, 1% TritonX-100, and 50% Glycerol.

10 x Taq-red reaction buffer: 500 mM KCl, 100 mM Tris-HCl (pH8.0), 20 mM $MgCl_2$.

Storage Conditions

Store the enzyme at -20˚C. The enzyme is stable for more than one year under suggested storage condition. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Protocol

The following is an example of PCR reaction, only for reference.

1. In a 0.2 ml or 0.5 ml thin wall tube, add the following components:

	20 µl reaction	50 µl reaction
10 x Taq-red reaction buffer	2 µl	5 µl
Forward primer	10-20 pmol	20-50 pmol
Reverse primer	10-20 pmol	20-50 pmol
Template DNA	5-50 ng	10-100 ng
dNTPs (10 mM each)	0.5 µl	1 µl
Taq-red DNA Polymerase	1-1.5 unit	2-3 units
ddH_2O	X µl	X µl
Total	20 µl	50 µl

2. Mix gently by pipetting, close the lid and centrifuge for a few seconds.
3. Add mineral oil to each tube (this step is unnecessary when using a thermal cycler with top heating).
4. Perform PCR cycles according to the PCR condition.
   (Annealing temperature and time need to be optimized for each primer/ template combination.)

94˚C	2.5 minutes	
94˚C	45 seconds	25-35 cycles
50-65˚C	1 minute	
72˚C	1-3 minutes	
72˚C	5-10 minutes	

5. Run 2~5 μl PCR products on 1% agarose gel stained with GoodView™ or EB.

f-Pfu DNA Polymerase

EP-500 500U

Description

f-Pfu DNA Polymerase is a thermostable enzyme with a molecular weight of 90 kDa. It catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction, resulting in blunt-ended PCR products without 3'-dA overhangs. f-Pfu DNA Polymerase exhibits 3'→5' exonuclease (proofreading) activity that enables the polymerase to correct mis-incorporated nucleotides, and lacks 5'→3' exonuclease activity. It is suitable for PCR and primer extension reaction that requires high fidelity when the PCR fragment is relatively shorter.

The extension rate of f-Pfu DNA Polymerase is about 1000 bp/min under standard condition. The optimal reaction temperature is 65~75℃, the optimal working concentration of dNTPs is 100~300 μM, the optimal working concentration of Mg^{2+} is 2~3 mM, and the suitable pH is 8.1~9.1. The amount of enzyme is 1~1.5 units for 20 μl PCR reaction, while 2~3 units for 50 μl PCR reaction.

Contents

	500 units
1. f-Pfu DNA Polymerase (5 units/μl)	100 μl
2. 10 x f-Pfu reaction buffer	1.5 ml
3. PCR dye	1.5 ml

f-Pfu DNA Polymerase Storage Buffer: f-Pfu DNA Polymerase is supplied in 50 mM Tris-HCl (pH8.2), 0.1 mM EDTA, 0.1% Tween20, 0.1% NP-40, 1 mM DTT and 50% Glycerol.

10 x f-Pfu reaction buffer: 100 mM KCl, 160 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 200 mM Tris-HCl (pH8.8), 1% TritonX-100, 1 mg/ml BSA (fraction V).

Storage Conditions

Store the components at -20℃. They are stable for more than one year under suggested storage condition. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Protocol

Note: PCR dye is an inert red dye, which greatly simplifies the operation procedure. The PCR products can be directly loaded to the agarose gel for electrophoresis with no need of additional loading buffer or tracking dye.

The following is an example of PCR reaction, only for reference.

1. In a 0.2 ml or 0.5 ml thin wall tube, add the following components:

	20 µl reaction	50 µl reaction
10 x f-Pfu reaction buffer	2 µl	5 µl
Forward primer	10-20 pmol	20-50 pmol
Reverse primer	10-20 pmol	20-50 pmol
Template DNA	5-50 ng	10-100 ng

dNTPs (10 mM each)	0.5 µl	1 µl
PCR dye (optional)	2 µl	5 µl
f-Pfu DNA Polymerase	1-1.5 unit	2-3 units
ddH_2O	X µl	X µl
Total	20 µl	50 µl

2. Mix gently by pipetting, close the lid and centrifuge for a few seconds.
3. Add mineral oil to each tube (this step is unnecessary when using a thermal cycler with top heating).
4. Perform PCR cycles according to the PCR condition. An example is given as follows:
   (Annealing temperature and time need to be optimized for each primer/template combination.)

94˚C	2.5 minutes	
94˚C	45 seconds	} 25-35 cycles
50-65˚C	1 minute	
72˚C	1-3 minutes	
72˚C	5-10 minutes	

5. Run 2~5 µl PCR products on 1% agarose gel stained with GoodView™ or EB.

Easy-Do™ PCR PreMix

EQ2.2-100 0.2ml thin-well tubes, 20ul reaction 100 tubes
EQ2.5-100 0.5ml thin-well tubes, 20ul reaction 100 tubes
EQ5.2-100 0.2ml thin-well tubes, 50ul reaction 100 tubes
EQ5.5-100 0.5ml thin-well tubes, 50ul reaction 100 tubes

Description

The Easy-Do™ PCR PreMix is a pre-mixed preparation in lyophilized format. The mix contains U-Taq DNA polymerase, reaction buffer, dNTPs, loading buffer and tracking dye for efficient PCR amplification. For reaction set-up, all you have to do is to add templates, specific primers and water. After PCR, the products can be directly loaded to the agarose gel without additional loading buffer or tracking dye. The Easy-Do™ PCR PreMix simplifies the procedure of PCR reaction and offers advantages of time-saving, convenience, consistency, and minimal risk of contamination.

Kit Contents

Easy-Do™ PCR PreMix 100 tubes

The optimal extension temperature of U-Taq DNA Polymerase in this kit is 70~75℃. The red tracking dye migrates at a rate between bromophenol blue and xylene cyanole, equivalent to the rate of 400~500bp fragment.

Each tube contains components as follows and is supplied in

a lyophilized format.

	20 µl reactions	50 µl reactions
U-Taq DNA Polymerase	1 unit	2 units
10×PCR reaction buffer	2 µl	5 µl
dNTPs (10 mM each)	200 µM each	500uM each
Loading dye	2 µl	5 µl
Stabilizer	2 µl	5µl

10 x U-Taq reaction buffer: 500 mM KCl, 100 mM Tris-HCl (pH8.4), 20 mM $MgCl_2$

Storage Conditions

Store at -20˚C. They are stable for more than one year under suggested storage condition.

Protocol

1. Add template DNA and primers to PCR thin wall tubes containing Easy-Do™ PCR PreMix.

	20 µl reactions	50 µl reactions
Template DNA	5-50 ng	10-100 ng
Forward primer	10-20 pmol	20-50 pmol
Reverse primer	10-20 pmol	20-50 pmol

2. Add ultrapure water to a total volume of 20 µl or 50 µl.
3. Dissolve the lyophilized red pellet by vortexing and briefly spin down.

4. Add mineral oil to PCR tubes (this step is unnecessary when using a thermal cycler with top heating).
5. Perform PCR reactions.
6. Load samples onto agarose gel without adding loading-dye buffer, and perform electrophoresis.

dNTPs Mix (10mM each)

EN-1 1 ml

10mM dNTPs Mix is a ready-to-use solution of dATP, dCTP, dGTP and dTTP (monosodium salts) at a concentration of 10mM each in sterile deionized water at pH7.5, whose purity is up to 99.5% (HPLC). It is free of RNase and DNase, and is suitable for any molecular biology application that requires pure deoxynucleotides, such as PCR, DNA sequencing, cDNA synthesis and nick translation.

Storage: Store at -20℃. They are stable for more than one year under suggested storage condition.

dNTPs Set (100 mM each)

EN-2 4×0.4 ml

dNTPs Set contains 4×0.4 ml of dATP, dCTP, dGTP and dTTP (monosodium salts) at a concentration of 100 mM each in sterile deionized water at pH7.5, whose purity is up to 99.5% (HPLC). It is free of RNase and DNase, and suitable for any molecular biology application that requires pure deoxynucleotides, such as PCR, DNA sequencing, cDNA synthesis and nick translation.

Storage: Store at -20℃. They are stable for more than one year under suggested storage condition.

全国统一热线：400 666 3029

sbs 赛百盛

Add: Room 202, Building 2, No. 1 Shangdi 4th Street,
Haidian District, Beijing 100085 China
Tel: +86-10-62969345/46, 82784296/92
Fax: +86-10-82784290
http://www.sbsbio.com
E-mail: info@sbsbio.com