Phusion DNA polymerase

Description:

Phusion DNA Polymerase is a chimeric Pfu which has a DNA binding protein at the N-terminal portion of the gene. This enzyme keeps significant activity after exposure to 99°C or repeated exposure to 98°C with more processivity and extention rate than Pfu DNA polymerase. It catalyzes the polymerization of nucleotides into duplex DNA in the 5'_3' direction, resulting in blunt-ended PCR products **without 3'-dA overhangs.**

Phusion DNA Polymerase exhibits 3'_5' exonuclease (proofreading) activity that enables the polymerase to correct the mis-incorporation of nucleotide, 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 **higher than 3kb.**

The enzyme exhibits 3´>5´ proofreading activity, resulting in over 20-fold higher PCR fidelity than possible with Taq DNA Polymerases.

Buffers and Reagents:

Storage Buffer: 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.5 mM EDTA, 0.1 mM DTT, 0.5% Tween 20,0.5 % Nonidet P-40, 50% Glycerol.

5X Reaction Buffer: Contains Tris-HCl (pH 9.0), PCR enhancers, KCl, (NH4)2SO4

Contents:

Components	C101101	C101102
Phusion DNA poly. 5 U/µl	**250 U**	**500 U**
$MgCl_2$ Solution 25 mM	**0.5 ml**	**1 ml**
5X Buffer $MgCl_2$ free	**1 ml**	**1 ml**

Kit storage: This kit should be stored at -20°C. Unnecessary repeated freeze/thawing should be avoided. Under these conditions reagents are stable for one year from the date of production.

General Reaction Protocol:

Please be sure to follow below

1. Thaw 10X reaction buffer, dNTP mixture.

Component	Vol (µL)
5X Reaction Buffer	4 µL
MgCl2 Solution 25mM	1.4 µL
40 mM dNTPs Mix (10 mM each)	0.5 µL
Upstream Primer (10 pmol/µl)	1 µL
Downstream Primer(10 pmol/µl)	1 µL
Phusion *DNA poly.* (5 units/µL)	0.25 µL
Template DNA	Variable
Sterilized D.W.	Variable
Total Volume	20 µL

2. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
3. Add templates DNA to the individual PCR tubes or wells containing the master mix.
5. Program the PCR machine according to the program outlined.

6. Place the PCR tubes or PCR plates in the terminal cycler and start the cycling program.

7. Separate the PCR products by agarose gel electrophoresis and visualize with EtBr or any other means.

Cycle	Time	Temp °C
1	30 Sec	98
25 ~35	10 Sec	98
	30 Sec	55~60
	30 ~60 Sec	72
1	5 Min	72

* Longer extension time makes nonspecific bands
*Extension rate for this enzyme is near 3000 bp/min.