molecular biology GENESPIN

DATA SHEET

info@genespin.com - www.genespin.com

> Pfu DNA Pol

cat. no.	*amount*	*note*
STS-P250	250 units	10X Standard Buffer
STS-P1000	1000 units	10X Standard Buffer
STS-Pw250	250 units	10X Standard Buffer w/o MgCl2
STS-Pw1000	1000units	10X Standard Buffer w/o MgCl2

add nucleotide box

cat. no.	*amount*	*note*
STSn-P250	250 units	10X Standard Buffer + dNTPs
STSn-P1000	1000 units	10X Standard Buffer + dNTPs
STSn-Pw250	250 units	10X Standard Buffer w/o MgCl2 + dNTPs
STSn-Pw1000	1000units	10X Standard Buffer w/o MgCl2 + dNTPs

Pfu DNA Pol is a superior thermostable DNA Pol isolated from an E.coli strain carrying the "pol" gene from Pyrococcus furiosus with 5' →3' polymerase activity and 3' → 5' exonuclease proofreading activity, i.e. it catalyzes DNA synthesis with very low error rate (about twelve times more accurate than Taq Pol). However, Pfu DNA Pol is slower than Taq Pol and typically requires about 1-2 min per cycle to amplify 1Kb of DNA.Using Pfu DNA Pol in PCR reactions also results in blunt-ended amplification products. Pfu DNA Pol is hence superior for techniques that require high fidelity DNA synthesis, but can also be used in conjunction with Taq Pol to obtain the fidelity of Pfu with the speed of Taq Pol activity.

FOR RESEARCH USE ONLY

UNIT DEFINITION
One unit is defined as the amount of enzyme required to incorporate 10 nanomoles of dNTPs into acid-insolubile material in 30 min at 74°C.

SHIPPING
Shipped in green ice.

STORAGE
Store at -20C°

SHELF LIFE
12 months

FORM
Liquid

CONCENTRATION
2U/ul

molecular biology GENESPIN

DATA SHEET

info@genespin.com - www.genespin.com

> Pfu DNA Pol

component	*STS-P250*	*STS-Pw250*	*STSn-P250*	*STSn-Pw250*
Pfu Polymerase	250 units / 125ul	250 units / 125ul	250 units / 125ul	250 units / 125ul
Standard Buffer	1ml 10X Buffer with MgCl2	1ml 10X Buffer w/o MgCl2	1ml 10X Buffer with MgCl2	1ml 10X Buffer w/o MgCl2
MgCl2	-	500ul / 50mM	-	500ul / 50mM
dNTPs	-	-	100ul / 10mM each	100ul / 10mM each

component	*STS-P1000*	*STS-Pw1000*	*STSn-P1000*	*STSn-Pw1000*
Pfu Polymerase	*1000* units / 500ul	*1000* units / 500ul	*1000* units / 500ul	*1000* units / 500ul
Standard Buffer	2ml 10X Buffer with MgCl2	2ml 10X Buffer w/o MgCl2	2ml 10X Buffer with MgCl2	2ml 10X Buffer w/o MgCl2
MgCl2	-	500ul / 50mM	-	500ul / 50mM
dNTPs	-	-	400ul / 10mM each	400ul / 10mM each

Assay Set-Up:

Before starting, vortex all components thoroughly to ensure homogeneity.
Prepare a premix for the number of assays you need according to the following protocol:

component	*stock conc.*	*final conc.*	*30ul reaction*
Standard Buffer	10X	1X	3.0ul
dNTPs	10mM each.	200uM	0.6ul
Pfu Polymerase	2U/ul	0.5U/ull	1.0ul
primers	1ug/ul each	50ng/ul each	2ul each
DNA Template	-	-	10-20ng
MG Water	-	-	up to 30ul

Cycling conditions:

Spin down the tubes/plate briefly to remove bubbles and place them into the cycler.

denaturation	95°C	5 min	1x
denaturation	95°C	45 sec - 1min	20-30x
annealing (1)	50-68°C	45 sec - 1min	
extension (2)	72°C	1 - 2 min	

1)The annealing temperature depends on the melting temperature of the primers used.
2)The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.