FS^{TM} Taq DNA Polymerase

Ref : P1071 250 U

Contents

	P1071
FS^{TM} *Taq* DNA Polymerase (5 U/µl)	50 µl
10X *FS*^{TM} PCR Buffer (Mg^{2+} Plus)	0.6 ml
6X Loading Buffer	0.25 ml

Note

• *FS*^{TM} *Taq* DNA Polymerase is 5 U/µl.

Default package is 5 U/µl.

• 10X *FS*^{TM} PCR Buffer (Mg^{2+} Plus) can replace with 10X PCR Buffer (Mg^{2+} free) and 25 mM $MgCl_2$. Please choose the appropriate package for your experiment.

Description

FS^{TM} Taq DNA polymerase is the latest generation Taq-based DNA polymerase. It possesses high amplification efficiency as Taq polymerase does, and fast elongation ability as *KOD* polymerase does, can be used in a variety of PCR. The *FS*^{TM} PCR Buffer , designed for *FS*^{TM} Taq DNA polymerase, can be used in fast amplification reaction. The elongation rate of *FS*^{TM} Taq DNA polymerase is 2-fold higher than the one of regular Taq DNA polymerase, which shortens the amplification time by half.

Features

- Fast rate of elongation: elongation velocity can reach to 3 kb/min, 2x higher than regular Taq DNA polymerase
- Highly thermostable : have a half-life of over 40 min at 95°C incubation
- Generates 3'-dA overhangs PCR products

Applications

- Routine PCR
- DNA labeling
- PCR sequencing
- Generate PCR product for TA cloning

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nM of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests.
Functionally tested in amplification of a single-copy gene from human genomic DNA.

Storage Buffer

20 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5 %TW 20, 0.5 % NP 40, 50 % Glycerol

10X *FS*^{TM} PCR Buffer

500 mM KCl, 100 mM Tris-Cl (pH 8.8), 15 mM $MgCl_2$, 1% Triton-100

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of FS^{TM} Taq DNA Polymerase primers, Mg^{2+} and template DNA) vary and need to be optimized.

For a total 50µl reaction volume

1. Add the following components to a sterile microcentrifuge tube sitting on ice:
   1.1. Recommended PCR assay with FS^{TM} PCR Buffer (Mg^{2+} plus)

Reagent	Quantity for 50µl of reaction mixture	Final Concentration
Sterile deionized water	variable	-
10X FS™ PCR Buffer (Mg^{2+} plus)	5 µl	1X
dNTPs (10mM each)	1 µl	0.2 mM each
Primer I	variable	0.4 - 1 µM
Primer II	variable	0.4 - 1 µM
FS™ Taq DNA Polymerase (5U/µl)	0.25 - 0.5 µl	1.25 - 2.5U/50 µl
Template DNA	variable	10pg-1µg

1.2. Recommended PCR assay with FS™ PCR Buffer (Mg^{2+} free)

Reagent	Quantity for 50µl of reaction mixture	Final Concentration
Sterile deionized water	variable	-
10X HS™ PCR Buffer (Mg^{2+} free)	5 µl	1X
dNTPs (10mM each)	1 µl	0.2 mM each
Primer I	variable	0.4 - 1 µM
Primer II	variable	0.4 - 1 µM
25 mM Mg^{2+}	variable	1 - 4 mM
FS™ Taq DNA Polymerase (5U/µl)	0.25 - 0.5 µl	1.25 - 2.5U/50 µl
Template DNA	variable	10pg-1µg

Table for selection of 25 mM $MgCl_2$ solution volume in 50 µl reaction mix :

Final Mg^{2+} conc.	1.0 mM	1.5 mM	2.0 mM	2.5 mM	3 mM	4 mM
Mg^{2+} Stock	2 µl	3 µl	4 µl	5 µl	6 µl	8 µl

Recommandations with Template DNA in a 50 µl reaction volume

Human genomic DNA	0.1 µg - 1 µg
Plasmid DNA	0.5 ng - 5 ng
Phage DNA	0.1 ng - 10 ng
E.coli genomic DNA	10 ng - 100 ng

2. Mix contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.
When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94°C	3 minutes
25-35 cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1 minute
Final extension	72°C	10 minutes

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Note:

- *FS*™ Taq DNA Polymerase is for High Specificity PCR applications.
- The half-life of enzyme is >40 minutes at 95°C.
- The error rate of *FS*™ Taq DNA Polymerase in PCR is 2.2×10^{-5} errors per nt per cycle; the accuracy (an inverse of the error rate) an average number of correct nucleotides incorporated before making an error, is 4.5×10^{-4} (determined according to the modified method described in).
- *FS*™ Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

Store all components at –20°C