Product information

Tsg Plus DNA polymerase

Catalog #:	D0088
Size:	200U, 5x200U
Concentration:	5U/µl
Storage:	-20°C

Description:

Tsg Plus is a mixture of Tsg and Proof reading enzyme. Tsg is a thermostable DNA polymerase isolated from a strain of Thermus sp (see product number D0081). Tsg Plus is used to improve the reliability and yield of conventional primer extension reaction.

10 x Tsg Plus reaction buffer:

200 mM TrisHCl (pH 8.8)
100 mM KCl
100 mM (NH4)2 SO4
20 mM Mg SO4
1% Triton X-100
1 mg / ml bovine serum albumin (BSA)

Reaction Conditions:

All reagents, including Tsg Plus, should be mixed immediately before use. DNA synthesis is performed in 100ul of mixture containing 20-200uM dNTPs, 0.3-1uM Primers, 0.1- 0.250ng of template DNA, 10ul of 10 x reaction buffer and 2.5-5 units of Tsg Plus. Mix the reaction gently, centrifuge briefly and then overlay with light mineral oil. Initially, denature the reaction by incubating at 95°C for 5 minutes and then cool to 40-68°C for 5 minutes to allow the primers to anneal to the template DNA.

Optimization of DNA synthesis:

It is important to add the reaction components in the following order:
1. H2O
2. 10 x reaction buffer
3. dNTPs
4. DNA template and primers
5. Tsg Plus

Reaction Mixture Set Up:

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Add components, in the following order, into a thin-walled PCR tube. Keep all components on ice.
   The following control PCR reactions should be run in parallel to ensure that the Tsg Plus DNA polymerase is working properly.

Reagent	Final Concentration	Quantity		Reagent	Positive Control	Negative Control
Water (PCR Grade)	-	variable		Water (PCR Grade)	32.8ul	33.8ul
10x Tsg Plus reaction buffer	1x	5ul		10x Tsg Plus reaction buffer	5ul	5ul
2.5mM dNTP mixture	200uM of each	4ul		2.5mM dNTP mixture	4ul	4ul
Primer I, forward	0.1-1uM	variable		Primer I(10µM), forward	1ul	1ul
Primer II, reverse	0.1-1uM	variable		Primer II(10µM), reverse	1ul	1ul
Tsg Plus DNA polymerase	1-1.5u/50ul	variable		Tsg Plus DNA polymerase	0.2ul	0.2ul
Template DNA	See note 1	variable		Control DNA Template	1ul	-
Total Volume	-	50ul		**Total Volume**	50ul	50ul

3. Gently vortex the sample and briefly centrifuge to collect all drops from walls of the tube.
4. Overlay the sample with one-half of the total reaction volume of mineral oil or add an appropriate amount of wax. This step may be omitted if the thermocycler is equipped with a heated lid.
5. Place samples in a thermocycler and start PCR.

Note for the Components of the Reaction Mixture:

1. **Template DNA:** Usually the amount of template DNA is in the range of 0.01-1ng plasmid or phage DNA and 0.1-1ug for genomic DNA, for a total reaction mixture of 50ul.
2. **Primers:** The PCR primers are usually 15-30 nucleotides in length, longer primers provide higher specificity. The GC content of primer should be 40-60%. The primer should not be self-complementary or complementary to any other primer in the reaction mixture, and the melting temperature of flanking primers should not differ by more than 5°C. If the primer is shorter than 25 nucleotides, the approx. melting temperature™ is calculated using the formula as: **Tm=4(G+C) + 2(A+T)**.
3. **dNTPs:** The final concentration of each dNTP in the reaction mixture is usually 200uM.
4. **Tsg plus DNA polymerase:** Usually 1-1.5u of Tsg plus DNA polymerase is used in the 50ul of reaction mix. Higher Tsg plus DNA polymerase concentrations may cause synthesis of nonspecific products. However, if inhibitors are present in the reaction mix (e.g., if the template DNA used is not highly purified), higher amounts of Tsg Plus DNA polymerase (2-3u) may be necessary to obtain a better yield of amplification products.
5. Usually the extending step is performed at 70-75°C. Tsg Plus DNA polymerase exhibits lower extension rate than Taq DNA polymerase, so 2min extension time is recommended for every 1 kb to be amplified.
6. **Cycling conditions:** Usually denaturation for 0.5-2min at 94-95°C is sufficient; the optimal annealing temperature is 5°C lower than the melting temperature of primer-template DNA duplex; Usually the extending step is performed at 70-75°C. Recommended extending time is 1min for the synthesis of PCR fragments up to 2kb. When larger DNA fragments are amplified, the extending time is usually increased by 1min for each 1kb.
7. **Number of cycles:** 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. For less than 10 copies of template DNA, 40 cycles should be performed. If the initial quantity of template DNA is higher, 25-35 cycles are usually sufficient.
8. **Final extending step:** After the last cycle, the samples are usually incubated at 72°C for 5-15min.

