Taq MutS

Code No.	316-04011
Size	50 µg
Concentration	1 µg/µl
Molecular Weight	89.3 kDa
Source	*Thermus aquaticus*
Storage Temperature	−20℃

Storage Condition

100 mM NaCl, 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 50% Glycerol

Enzyme Reaction Condition

100 mM KCl, 50 mM Tris-HCl (pH 8.5 at 25℃), 20 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 2% Glycerol
Reaction Temperature: 65℃

■ The attached exclusive buffer for the enzyme reaction (**Store at -20℃**)

・10 × *Taq* MutS Buffer 1 ml

It is 10-fold concentrated compared to the enzyme reaction condition.

Binding test:
In 20 µl of the reaction solution, 0.5 µg of the product can bind to not less than 50% of 100 ng of a 36 bp synthetic DNA having 1 base deletion at 65°C for 30 min.

Purity:
Three µg of the product and 0.5 µg of plasmid pBR322 were reacted at 37°C for 16 hr and then agarose gel (0.8% Agarose S) electrophoresis was performed. The result indicated no increase of oc-DNA.

Three µg of the product and 0.5 µg of λDNA were reacted at 37°C for 16 hr and then agarose gel (0.8% Agarose S) electrophoresis was performed. The result indicated no degradation of λDNA.

Three µg of the product and 2 µg of substrate RNA were reacted at 37°C for 16 hr and then agarose gel (2% Agarose S) electrophoresis was performed. The result indicated no degradation of RNA.

References

1) Biswas, I. and Hsieh, P.: *J. Biol. Chem.*, 271 (9), 5040-5048 (1996)
2) Biswas, I. and Hsieh, P.: *J. Biol. Chem.*, 272 (20), 13355-13364 (1997)
3) Takamatsu, S., Kato, R. and Kuramitsu, S.: *Nucleic Acids Res.*, 24 (4), 640-647 (1996)
4) Whitehouse, A., Deeble, J., Parmar, R., Taylor, G. R., Markham, A. F. and Meredith, D. M.: *Biochem. Biophys. Res. Commun.*, 233, 834-837 (1997)
5) Lishanski, A., Ostrander, E. A. and Rine, J.: *Proc. Natl. Acad. Sci. USA*, 91, 2674-2678 (1994)
6) Wagner, R., Debbie, P. and Radman, M.: *Nucleic Acids Res.*, 23 (19), 3944-3948 (1995)

Example of use

Test for detecting the deleted region using PCR products

A plasmid having a mutated sequence (M: mutant) in which 2 nucleotides are deleted as compared to the normal sequence (N: normal) was produced. Respective PCRs were performed using N or M singly, or a mixture of N and M as templates. After completing PCR, the samples were denatured and annealed, and reacted by directly adding *Taq* MutS (1 µg) at 65 °C for 30 min, and then subjected to electrophoresis. PCR was performed so that the lengths of the amplified products were 3 kinds of 60 bp, 100 bp and 200 bp, and the deleted region was detected in each of the products.

Template DNA (N/M, N or M)	1 µl
10 × Gene *Taq* Universal Buffer	5 µl
dNTP Mixture (2.5 mmol/l each)	4 µl
Primer-forward (20 pmol/µl)	1 µl
Primer-reverse (20 pmol/µl)	1 µl
Gene *Taq* (5 units/µl)	0.5 µl
H_2O	37.5 µl
Total	50 µl

↓

94℃ 1 min.
94℃ 10 sec.
60℃ 15 sec. } 25 cycles
72℃ 20 sec.
94℃ 5 min. (denaturation of PCR products)
72℃ 30 min. (re-annealing)
4℃

↓

PCR products 10 µl ＋ *Taq* MutS 1 µl (1 µg/µl)

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65℃, 30 min.

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＋ 2 µl Loading buffer
(0.02% Bromophenol blue, 0.02% Xylene cyanol FF, 50% Glycerol, 200 mM Tris-HCl pH 7.5)

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Electrophoresis in polyacrylamide gradient gel

Polyacrylamide gradient gel
- 4－10% native polyacrylamide
- 1 × TAE
- 0.1 mM $MgCl_2$

Electrophoresis Running buffer
- 1 × TAE
- 0.1 mM $MgCl_2$

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Stain the gel with SYBR® Gold

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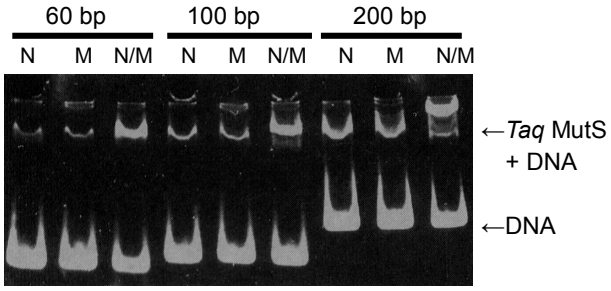

(For more details on experimental examples and the like, see the website of Nippon Gene.)

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