Product Name: T7 Endonuclease I

日本語データシート

Code: DS715

Size: 50 reactions

Research Use Only

Kit Contents:

Component	Quantity	Storage
①10× T7 Endonuclease I	1 Tube	-20℃
②10× Digestion Buffer	1 Tube	-20℃
③Digestion Control	1 Tube	-20℃

Storage condition:

Store at -20℃

Stable for 12 months from the date of receipt

※③Digestion Control may form sediment. In such cases, use the supernatant after centrifugation.

Introduction:

T7 Endonuclease I is a mismatch DNA cleavage enzyme that detect the editing efficiency of genomic DNA. Loci amplicons where the gene-specific double-strand breaks occur is denatured and reannealed to generate mismatches. The mismatches are cleaved by T7 Endonuclease I and then the resultant bands are analyzed by gel electrophoresis.

Required materials not included with the kit:

・Thermal cycler
・Block heater
・Tubes, Micro Pipettes & Tips
・Agarose gel, Electrophoresis system & Analysis software
・Nuclease free water (e.g. DR120)

Methods

Cleavage assay:

1. Combine following samples for re-annealing.

	Sample	Digestion Control
PCR Product	1–8 μL	1 μL
10 × Digestion Buffer	1 μL	1 μL
Water up to	9 μL	9 μL

* Optimal input depends on the sample. Adjust the sample amount for agarose gel electrophoresis detection.

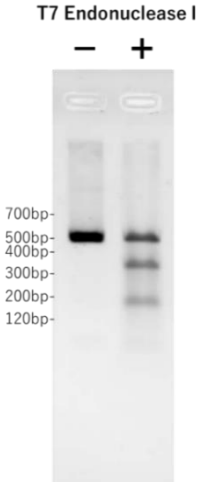
2. Place the PCR tube from above in a thermal cycler with a heated-lid and run the following program.

Stage	Temp	Time
1	95℃	5 min
2	95–85℃	−2℃/sec
3	85–25℃	−0.1℃/sec
4	4℃	Hold

3. Add 1 μL 10× T7 Endonuclease I to test samples and mix well.
4. Incubate at 37°C for 15 min.
5. Place the PCR tubes at 4°C immediately to reduce the overreaction.
6. Run agarose gel electrophoresis.

（If not analyzed immediately, add 1 μL of 100 μM EDTA to each reaction and store samples at -20°C.）

Clevage of control:



3% Agarose Gel Electrophoresis