The full-length, active T7 Endo I is generated in vitro by ligating a synthetic peptide, consisting of the truncated amino acid residues, to the thromine-tagged tT7 Endo I (1).

**Applications:**
- Resolve four-way junction or branched DNA
- Detect or cleave heteroduplex and nicked DNA
- Randomly cleave linear DNA for shot-gun cloning

**Reaction Conditions:** 1X NEBuffer 2. Incubate at 37°C.

**Quality Assurance:** Purified free of contaminating exonucleases and endonucleases.

**Enzyme Properties**

**Activity in NEBuffers:**
- NEBuffer 1: 50%
- NEBuffer 2: 100%
- NEBuffer 3: 100%
- NEBuffer 4: 100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Notes On Use:** 10–15 units will cut 1 µg pUC19 in 4 hours resulting in equal amounts of linear and nicked DNA.

---

**Quality Control Assays**

**Ligation:** 16 µg of Hae III digested φX174 DNA was incubated with 40 units of T7 Endonuclease I in a total reaction volume of 400 µl for 2 hours at 37°C. The DNA was ethanol precipitated and ligated with T4 DNA Ligase and then recut with Hae III. Approximately 50% of the T7 Endo I treated fragments were ligated and of those, > 95% were recut with Hae III.

---

**Enzyme Properties**

**Activity in NEBuffers:**
- NEBuffer 1: 50%
- NEBuffer 2: 100%
- NEBuffer 3: 100%
- NEBuffer 4: 100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Notes On Use:** 10–15 units will cut 1 µg pUC19 in 4 hours resulting in equal amounts of linear and nicked DNA.

---

**Quality Control Assays**

**Ligation:** 16 µg of Hae III digested φX174 DNA was incubated with 40 units of T7 Endonuclease I in a total reaction volume of 400 µl for 2 hours at 37°C. The DNA was ethanol precipitated and ligated with T4 DNA Ligase and then recut with Hae III. Approximately 50% of the T7 Endo I treated fragments were ligated and of those, > 95% were recut with Hae III.

---

**Enzyme Properties**

**Activity in NEBuffers:**
- NEBuffer 1: 50%
- NEBuffer 2: 100%
- NEBuffer 3: 100%
- NEBuffer 4: 100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Notes On Use:** 10–15 units will cut 1 µg pUC19 in 4 hours resulting in equal amounts of linear and nicked DNA.

---

**Quality Control Assays**

**Ligation:** 16 µg of Hae III digested φX174 DNA was incubated with 40 units of T7 Endonuclease I in a total reaction volume of 400 µl for 2 hours at 37°C. The DNA was ethanol precipitated and ligated with T4 DNA Ligase and then recut with Hae III. Approximately 50% of the T7 Endo I treated fragments were ligated and of those, > 95% were recut with Hae III.

---

**Enzyme Properties**

**Activity in NEBuffers:**
- NEBuffer 1: 50%
- NEBuffer 2: 100%
- NEBuffer 3: 100%
- NEBuffer 4: 100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Notes On Use:** 10–15 units will cut 1 µg pUC19 in 4 hours resulting in equal amounts of linear and nicked DNA.
T7 Endonuclease I is a structure-selective enzyme. It acts on a variety of DNA substrates with different specific activities. It is important to control the amount of enzyme and the reaction time used for cleavage of a particular substrate.

This enzyme is not recommended to be used at 55°C, as the activity is decreased.

References: