DNA Polymerase I (E. coli)

**Applications:**
- Nick translation of DNA to obtain probes with a high specific activity (2)
- Second strand synthesis of cDNA (3,4)

Supplied in: 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

**Reagents Supplied with Enzyme:**
- 10X NEBuffer 2.

**Reaction Conditions:** 1X NEBuffer 2. Supplement with dNTPs (not included). Incubate at 37°C.

DNA Polymerase I (E. coli) is active in all four NEBuffers when supplemented with dNTPs.

1X NEBuffer 2:
- 50 mM NaCl
- 10 mM Tris-HCl
- 10 mM MgCl₂
- 1 mM DTT
- pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.

Unit Assay Conditions: 1X NEBuffer 2, 33 µM dNTPs including [32P]-dTTP and 70 µg/ml denatured herring sperm DNA.

Molecular Weight: 109,000 daltons.

Notes On Use: DNase I is not included with this enzyme and must be added for nick translation reactions.

References:

Companion Products Sold Separately:
- NEBuffer 2
  - #B7002S 6.0 ml
- DNase I (RNase-free)
  - #M0303S 1,000 units
  - #M0303L 5,000 units
- Deoxynucleotide Solution Set
  - #N0446S 25 µmol of each
- Deoxynucleotide Solution Mix
  - #N0447S 8 µmol of each
  - #N0447L 40 µmol of each

**References:**

DNA Polymerase I (E. coli)

**Applications:**
- Nick translation of DNA to obtain probes with a high specific activity (2)
- Second strand synthesis of cDNA (3,4)

Supplied in: 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

**Reagents Supplied with Enzyme:**
- 10X NEBuffer 2.

**Reaction Conditions:** 1X NEBuffer 2. Supplement with dNTPs (not included). Incubate at 37°C.

DNA Polymerase I (E. coli) is active in all four NEBuffers when supplemented with dNTPs.

1X NEBuffer 2:
- 50 mM NaCl
- 10 mM Tris-HCl
- 10 mM MgCl₂
- 1 mM DTT
- pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.

Unit Assay Conditions: 1X NEBuffer 2 containing a minimum of 25 units of DNA Polymerase I (E. coli) with 1 µg of supercoiled φX174 DNA for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Molecular Weight: 109,000 daltons.

Notes On Use: DNase I is not included with this enzyme and must be added for nick translation reactions.

References:

Companion Products Sold Separately:
- NEBuffer 2
  - #B7002S 6.0 ml
- DNase I (RNase-free)
  - #M0303S 1,000 units
  - #M0303L 5,000 units
- Deoxynucleotide Solution Set
  - #N0446S 25 µmol of each
- Deoxynucleotide Solution Mix
  - #N0447S 8 µmol of each
  - #N0447L 40 µmol of each

**References:**