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04 Aug 2015

Date

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New England Biolabs Product Specification

Product Name: TherminatorTM DNA Polymerase

Catalog #: M0261S/L

Concentration: 2,000 units/ml

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 75°C

Shelf Life: 24 months
Storage Temp: -20°C

Storage Conditions: 10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)

Specification Version: PS-M0261S/L v1.0

Effective Date: 04 Aug 2015

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 μ l reaction in ThermoPol® Reaction Buffer containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 20 units of TherminatorTM DNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 μ l reaction in ThermoPol® Reaction Buffer containing 1 μ g of a mixture of single and double-stranded [3 H] *E. coli* DNA and a minimum of 20 units of TherminatorTM DNA Polymerase incubated for 4 hours at either 37° C or 75°C releases <0.1% of the total radioactivity.

Non-Specific DNase Activity (16 Hour) - A 50 μl reaction in NEBuffer 2 containing 1 μg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 2 units of TherminatorTM DNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM *p*-Nitrophenol Phosphate (pNPP) and a minimum of 100 units TherminatorTM DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Protein Purity Assay (SDS-PAGE) - TherminatorTM DNA Polymerase is $\geq 98\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion) - A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 μ l of TherminatorTM DNA Polymerase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Derek Robinson

Director of Quality Control





