

New England Biolabs Product Specification

<i>Product Name:</i>	<i>dUTP Solution</i>
<i>Catalog #:</i>	<i>N0459S</i>
<i>Concentration:</i>	<i>100 mM</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>Supplied in Ultrapure water as a sodium salt (pH 7.5)</i>
<i>Specification Version:</i>	<i>PS-N0459S v2.0</i>
<i>Effective Date:</i>	<i>16 Jan 2022</i>

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in NEBuffer 2 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 µl of dUTP Solution incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 4 µl of dUTP Solution incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

PCR Amplification (0.5 kb Lambda, dNTPs) - A 50 µl reaction in ThermoPol® Reaction Buffer in the presence of 200 µM dATP, dGTP, dCTP, and dUTP and 0.2 µM primers containing 1 ng Lambda DNA with 1.25 units of *Taq* DNA Polymerase for 25 cycles of PCR amplification results in the expected 0.5 kb product.

PCR Amplification (2.0 kb Lambda, dNTPs) - A 50 µl reaction in ThermoPol® Reaction Buffer in the presence of 200 µM dATP, dGTP, dCTP, and dUTP and 0.2 µM primers containing 1 ng Lambda DNA with 1.25 units of *Taq* DNA Polymerase for 25 cycles of PCR amplification results in the expected 2.0 kb product.

Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 16 µl of dUTP Solution incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Physical Purity (HPLC) - dUTP Solution is ≥ 99% pure as determined by HPLC analysis.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 1 µl of dUTP Solution is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.



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RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of dUTP Solution 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.

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Date 16 Jan 2022

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