

New England Biolabs Product Specification

<i>Product Name:</i>	NEBNext [®] Second Strand Synthesis (dNTP-free) Reaction Buffer
<i>Catalog #:</i>	B6117S
<i>Concentration:</i>	10X Concentrate
<i>Shelf Life:</i>	24 months
<i>Storage Temp:</i>	-20°C
<i>Composition (1X):</i>	20 mM Tris-HCl, 12 mM (NH ₄) ₂ SO ₄ , 5 mM MgCl ₂ , 0.16 mM β-NAD, (pH 7.5 @ 25°C)
<i>Specification Version:</i>	PS-B6117S v2.0
<i>Effective Date:</i>	12 Feb 2020

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking, Buffer) - A 50 µl reaction in 1X NEBNext[®] Second Strand Synthesis (dNTP-free) Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA 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, Buffer) - A 50 µl reaction in 1X NEBNext[®] Second Strand Synthesis (dNTP-free) Reaction Buffer containing 1 µg of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA 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, Buffer) - A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM MgCl₂ containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 20 µl NEBNext[®] Second Strand Synthesis (dNTP-free) Reaction Buffer incubated for 4 hours at 37°C yields <0.00001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

RNase Activity (Buffer) - A 10 µl reaction in 1X NEBNext[®] Second Strand Synthesis (dNTP-free) Reaction Buffer containing 40 ng of a 300 base single-stranded RNA 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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