NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*NEBNext® Second Strand Synthesis (dNTP-free) Reaction Buffer*
Catalog Number:	*B6117S*
Concentration:	*10 X Concentrate*
Packaging Lot Number:	*10064428*
Expiration Date:	*09/2021*
Storage Temperature:	*-20°C*
Specification Version:	*PS-B6117S v2.0*
Composition (1X):	*20 mM Tris-HCl, 12 mM (NH4)2SO4, 5 mM MgCl2, 0.16 mM β-NAD, (pH 7.5 @ 25°C)*

NEBNext® Second Strand Synthesis (dNTP-free) Reaction Buffer Component List

NEB Part Number	Component Description	Lot Number	Individual QC Result
B6117SVIAL	NEBNext® Second Strand Synthesis (dNTP-free) Reaction Buffer	10061496	**Pass**

Assay Name/Specification	Lot # 10064428
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.	**Pass**
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.	**Pass**
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.	**Pass**
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.	**Pass**

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This product has been tested and shown to be in compliance with all specifications.

Melissa Arn
Production Scientist
26 Nov 2019

Josh Hersey
Packaging Quality Control Inspector
16 Mar 2020