

## 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:** 10187033  
**Expiration Date:** 04/2025  
**Storage Temperature:** -20°C  
**Specification Version:** PS-B6117S v2.0  
**Composition (1X):** 20 mM Tris-HCl, 12 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 5 mM MgCl<sub>2</sub>, 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	10187034	Pass

Assay Name/Specification	Lot # 10187033
<b>Endonuclease Activity (Nicking, Buffer)</b> 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
<b>Non-Specific DNase Activity (16 hour, Buffer)</b> 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
<b>Phosphatase Activity (pNPP, Buffer)</b> A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM MgCl <sub>2</sub> 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
<b>RNase Activity (Buffer)</b> 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

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

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Christine Sumner  
Production Scientist  
15 May 2023



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Michael Tonello  
Packaging Quality Control Inspector  
22 May 2023