

New England Biolabs Product Specification

<i>Product Name:</i>	<i>NEBuilder[®] HiFi DNA Assembly Cloning Kit</i>
<i>Catalog #:</i>	<i>E5520S</i>
<i>Kit Components:</i>	<i>NEBuilder[®] HiFi DNA Assembly Master Mix (M5520) — Store at -20°C</i> <i>NEBuilder[®] Positive Control (N2611) — Store at -20°C</i> <i>NEB[®] 5-alpha Competent E. coli (High Efficiency) (C2987) — Store at -80°C</i> <i>SOC Outgrowth Medium (B9020) — Store at 4°C</i> <i>pUC19 Vector (N3041) — Store at -20°C</i>
<i>Shelf Life:</i>	<i>12 months</i>
<i>Storage Temp:</i>	<i>Multi-temperature</i>
<i>Specification Version:</i>	<i>PS-E5520S v3.0</i>
<i>Effective Date:</i>	<i>15 May 2024</i>

Assay Name/Specification (minimum release criteria)

Functional Testing (NEBuilder[®] HiFi DNA Assembly) - 10 µl of 2X NEBuilder[®] HiFi DNA Assembly Cloning Kit was incubated with 0.05 pmol each of 6 DNA fragments (4 fragments of 1,000 bp, one fragment of 1,152 bp with 80 bp overlap, and a vector of 3,373 bp with a 20 bp overlap) in a final volume of 20 µl at 50°C for 60 minutes. NEB[®] 5-alpha Competent *E. coli* (High Efficiency) were transformed with 2 µl of the assembled products. Successfully assembled fragments produce an intact lacZ gene in the pACYC184 vector, and yield blue colonies on an IPTG/Xgal/Chloramphenicol plate when incubated overnight at 37°C after transformation. Greater than 100 blue colonies were observed when 1/10 of the outgrowth (500 µl) was spread on a plate.

* **Individual Product Component Note** - Standard Quality Control Tests are performed for each component included in NEBuilder[®] HiFi DNA Assembly Cloning Kit and meet the designated specifications.

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Date 15 May 2024

Nancy Considine
Quality Approver

