

New England Biolabs Product Specification

Product Name: SHuffle[®] T7 Competent *E. coli*
Catalog #: C3026J
Shelf Life: 12 months
Storage Temp: -80°C
Specification Version: PS-C3026J v2.0
Effective Date: 01 May 2023

Assay Name/Specification (minimum release criteria)

Antibiotic Resistance (Nitrofurantoin) - 15 µl of untransformed SHuffle[®] T7 Competent *E. coli* streaked onto a LB or Rich Broth plate containing Nitrofurantoin will form colonies after incubation for 16 hours at 37°C.

Antibiotic Resistance (Spectinomycin) - 15 µl of untransformed SHuffle[®] T7 Competent *E. coli* streaked onto a LB or Rich Broth plate containing Spectinomycin will form colonies after incubation for 16 hours at 37°C.

Antibiotic Resistance (Streptomycin) - 15 µl of untransformed SHuffle[®] T7 Competent *E. coli* streaked onto a LB or Rich Broth plate containing Streptomycin will form colonies after incubation for 16 hours at 37°C.

Antibiotic Sensitivity (Ampicillin) - 15 µl of untransformed SHuffle[®] T7 Competent *E. coli* streaked onto a LB or Rich Broth plate containing Ampicillin will not form colonies after incubation for 16 hours at 37°C.

Antibiotic Sensitivity (Chloramphenicol) - 15 µl of untransformed SHuffle[®] T7 Competent *E. coli* streaked onto a LB or Rich Broth plate containing Chloramphenicol will not form colonies after incubation for 16 hours at 37°C.

Antibiotic Sensitivity (Kanamycin) - 15 µl of untransformed SHuffle[®] T7 Competent *E. coli* streaked onto a LB or Rich Broth plate containing Kanamycin will not form colonies after incubation for 16 hours at 37°C.

Antibiotic Sensitivity (Tetracycline) - 15 µl of untransformed SHuffle[®] T7 Competent *E. coli* streaked onto a LB or Rich Broth plate containing Tetracycline will not form colonies after incubation for 16 hours at 37°C.

Functional Testing (Disulfide Bond Formation) - The nuclease NucA requires disulfide bonds for its stability. When expressed at 30°C in *E. coli*, NucA is toxic to cells only in its oxidized disulfide-bonded state. Transformation of SHuffle[®] T7 Express Competent *E. coli* using 100 pg of plasmid that expresses a MBP-NucA fusion results in < 1% of the colonies when compared to a control transformation of its wild type parent strain NEB 10-beta after 0.4 mM IPTG induction at 30°C.

Phage Resistance (Φ 80) - 15 µl of untransformed SHuffle[®] T7 Competent *E. coli* streaked onto a Rich Broth plate does not support plaque formation by phage Φ 80 after incubation for 16 hours at 37°C.



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Transformation Efficiency - 50 µl of SHuffle [®] T7 Competent <i>E. coli</i> cells were transformed with 100 pg of pUC19 DNA using the transformation protocol provided. Incubation overnight on LB-Ampicillin plates at 37°C resulted in >1 x 10 ⁶ cfu/µg of DNA.

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Date 01 May 2023

Lauren Brown
Quality Approver

