

## New England Biolabs Product Specification

*Product Name:* N1-Methyl-Pseudouridine-5'-Triphosphate (N1-Methyl-Pseudo-UTP)  
*Catalog #:* N0431S  
*Concentration:* 100 mM  
*Shelf Life:* 24 months  
*Storage Temp:* -20°C  
*Storage Conditions:* Supplied as sodium salts, aqueous solution, pH 7.4  
*Specification Version:* PS-N0431S v1.0  
*Effective Date:* 17 Jul 2024

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking)** - A 50 µl reaction in NEBuffer 4 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 500 nmol of N1-Methyl-Pseudouridine-5'-Triphosphate incubated for 16 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Functional Testing (High Yield RNA Synthesis using Modified Nucleotides)** - A 20 µl reaction in transcription reaction buffer containing 1 µg CLuc Control Template, 2 µl T7 RNA Polymerase Mix, and 10 mM ribonucleotides (each) incubated for 2 hours at 37°C yields ≥ 150 µg RNA transcript. Only one modified NTP is assayed per reaction. The modified NTP is fully substituted in place of the unmodified counterpart NTP.

**Physical Purity (HPLC)** - N1-Methyl-Pseudouridine-5'-Triphosphate is ≥ 95% pure as determined by HPLC analysis.

**RNase Activity (Extended Digestion)** - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 100 nmol of N1-Methyl-Pseudouridine-5'-Triphosphate 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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Quality Approver

