

New England Biolabs Product Specification

<i>Product Name:</i>	SP6 RNA Polymerase
<i>Catalog #:</i>	M0207S/L
<i>Concentration:</i>	20,000 units/ml
<i>Unit Definition:</i>	One unit is defined as the amount of enzyme required to incorporate 1 nmol ATP into an acid-insoluble material in 1 hour at 37°C.
<i>Shelf Life:</i>	24 months
<i>Storage Temp:</i>	-20°C
<i>Storage Conditions:</i>	50 mM Tris-HCl, 100 mM NaCl, 20 mM βME, 1 mM EDTA, 0.1 % Triton®X-100, 50 % Glycerol, (pH 7.9 @ 25° C)
<i>Specification Version:</i>	PS-M0207S/L v1.0
<i>Effective Date:</i>	03 May 2018

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of SP6 RNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 100 units of SP6 RNA Polymerase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of SP6 RNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Promoter Specificity - A 50 µl reaction in RNAPol Reaction Buffer in the presence of 2 mM NTPs containing 1 µg of Lambda DNA as a template and a minimum of 100 units of SP6 RNA Polymerase incubated for 1 hour at 37°C results in <1.5% of the amount of product incorporated as compared to a control reaction using SP6 DNA as a template.

Protein Purity Assay (SDS-PAGE) - SP6 RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion) - A 10 µl reaction in RNAPol Reaction Buffer containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of SP6 RNA Polymerase is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.



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Date 03 May 2018

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