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New England Biolabs
Product Specification

Product Name:	*WarmStart® Nt.BstNBI*
Catalog #:	*R0725S*
Concentration:	*10,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to digest 1 µg T7 DNA in NEBuffer r3.1 in 1 hour at 55°C in a total reaction volume of 50 µl.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)*
Specification Version:	*PS-R0725S v1.0*
Effective Date:	*20 May 2022*

Assay Name/Specification (minimum release criteria)

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 50 units of WarmStart® Nt.BstNBI incubated for 4 hours at 55°C releases <0.1% of the total radioactivity.

Functional Testing (WarmStart Inhibition) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of T7 DNA and a minimum of 10 units of WarmStart® Nt.BstNBI incubated for 1 hour at 25°C results in <5% digestion of the DNA as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16 hour) - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of T7 DNA and a minimum of 10 units of WarmStart® Nt.BstNBI incubated for 16 hours at 55°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. NOTE: although no nuclease degradation is detected under these conditions, extended incubations and/or high concentrations of this enzyme may result in star activity. See the product FAQ for recommended reaction conditions for this enzyme.

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

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 10 units of WarmStart® Nt.BstNBI is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.

Date 20 May 2022

Derek Robinson
Director, Quality Control