

## New England Biolabs Product Specification

Product Name:	<i>Φ29 DNA Polymerase Reaction Buffer</i>
Catalog #:	B0269S
Concentration:	10X Concentrate
Shelf Life:	60 months
Storage Temp:	-20°C
Composition (1X):	50 mM Tris-HCl, 10 mM MgCl <sub>2</sub> , 10 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 4 mM DTT, (pH 7.5 @ 25°C)
Specification Version:	PS-B0269S v1.0
Effective Date:	29 Sep 2016

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

**Endonuclease Activity (Nicking, Buffer)** - A 50 µl reaction in 2X phi29 DNA Polymerase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Non-Specific DNase Activity (16 hour, Buffer)** - A 50 µl reaction in 2X phi29 DNA Polymerase Reaction Buffer containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**pH (buffers/solutions)** - The pH of 10X phi29 DNA Polymerase Reaction Buffer is between pH 7.4 and 7.6 at 25°C.

**Phosphatase Activity (pNPP, Buffer)** - A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub> containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 40 µl phi29 DNA Polymerase Reaction Buffer incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 1 µl of phi29 DNA Polymerase Reaction Buffer 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.

**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 1 µl of phi29 DNA Polymerase Reaction Buffer 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.



Date 29 Sep 2016

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