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ProductInformation

Chloroform
PCR Reagent

Product Number **C 7559**
Store At Room Temperature

Product Description
RNase, DNase: None detected
Purity: 99+%
Suitable for use in the Polymerase Chain Reaction (PCR) (see comments section)

PCR Suitability
At the conclusion of a 25 cycle PCR, to facilitate recovery of the aqueous phase, 100 µl of chloroform was added to a 100 µl PCR mixture overlaid with 100 µl mineral oil. The mixture was briefly mixed using a vortex mixer and then centrifuged for 30 seconds at 14000 x g. It was observed that the aqueous phase was floating near the surface of the chloroform-oil mixture. Upon analysis of the PCR product, no detectable degradation or interference with electrophoresis was observed.

The PCR was performed according to the following conditions: A reaction mixture containing 10 mM Trizma®-HCl, pH 8.3 at 25 °C, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001%(w/v) gelatin, each dNTP at 200 µM, primers defining an approximately 500 base pair region of λ DNA at 1.0 µM each, λ DNA template at 1 ng/100 µl, and *Taq* DNA polymerase at 2.5 units/100 µl. The reaction underwent 25 cycles of 94 °C to denature the double stranded DNA, 55 °C to anneal the DNA segments, and 72 °C to extend the DNA segments. A single band of approximately 500 base pairs was visualized following electrophoresis of the reaction product in a 1.5% agarose gel.

Endonuclease-Exonuclease
A 50 µl reaction containing one µg of λ DNA Hind III digest, 30 mM Trizma®-HCl, pH 7.8, 50 mM NaCl and 10 mM $MgCl_2$ was extracted with 100 µl chloroform as follows. The mixture was briefly mixed using a vortex mixer and then centrifuged at 14,000 x g for 30 seconds. The upper aqueous phase was removed and incubated at 37 °C for 16 hours. No degradation of the DNA fragments was detected by agarose gel electrophoresis. Detection limit: Degradation of 10% of the DNA substrate is detectable.

Endonuclease (Nickase)
A 50 µl reaction containing one µg of pBR322, 30 mM Trizma®-HCl, pH 7.8, 50 mM NaCl and 10 mM $MgCl_2$ was extracted with 100 µl chloroform as follows. The mixture was briefly mixed using a vortex mixer and then centrifuged at 14,000 x g for 30 seconds. The upper aqueous phase was removed and incubated at 37 °C for 16 hours. No conversion of the covalently closed circular DNA to the nicked or linear form was observed by agarose gel electrophoresis. Detection limit: Conversion of 1% of the DNA substrate is detectable.

RNase
A 50 µl reaction containing two µg of tRNA, 30 mM Trizma®-HCl, pH 7.8, 50 mM NaCl and 10 mM $MgCl_2$ was extracted with 100 µl chloroform as follows. The mixture was briefly mixed using a vortex mixer and then centrifuged at 14,000 x g for 30 seconds. The upper aqueous phase was removed and incubated at 37 °C for 16 hours. No degradation of the tRNA was detected by polyacrylamide gel electrophoresis. Detection limit: Degradation of 10% of the tRNA substrate is detectable.

Comments
The PCR process is covered by patents owned by Hoffmann-La Roche, Inc. Purchase of these products does not convey a license under these patents. Information about licenses to PCR can be obtained from The Perkin-Elmer Corporation or Roche Molecular Systems, Inc.

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