ThermoFisher SCIENTIFIC

Certificate of Analysis

Zero Blunt™ TOPO™ PCR Cloning Kit for Sequencing, without competent cells

Product No. 450031, 11533837

Lot No. 2880778

Date of Manufacture 21-Feb-2024

Restriction Enzyme Analysis

The parental supercoiled plasmid is qualified by restriction digest to confirm its identity prior to linearization and adaptation with topoisomerase I. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The table below lists the restriction enzymes and the expected fragments.

Restriction Enzyme	Expected Fragments (bp)
Pme I	3957 (linearize)
Xba I	3957 (linearize)
*Bsp*H I	1008, 2949
Fsp I	899, 1132, 1926
EcoR I and *Afl* III	16, 408, 716, 2789

Note: The *Xba* I site is removed during the linearization and adaptation process and is not present in the sequence of the linearized, adapted vector.

Results: Meets specification

TOPO Cloning

Each lot is qualified using control reagents included in the Zero Blunt® TOPO® PCR Cloning Kit for Sequencing. Under conditions described in the on-line manual, a 750 bp control PCR product is amplified and TOPO® Cloned into pCR®4 Blunt-TOPO® vector and subsequently transformed into the One Shot® competent *E. coli*. The following results were obtained:

1) ≥ 95% cloning efficiency when vector + PCR insert colonies are analyzed by *EcoR* I digestion and agarose gel electrophoresis.

2) ≤ 5% of foreground will be produced in the vector-only reaction.

Results: Meets specification

Sequencing Primers

Sequencing primers are lot-qualified by DNA sequencing experiments using the dideoxy chain termination technique. Each primer must yield ≥ 250 bp of quality sequence from a supercoiled plasmid template using standard sequencing conditions.

Results: Meets specification

For Research Use Only. Not for use in diagnostic procedures.

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