

# Certificate of Analysis

## Zero Blunt™ TOPO™ PCR Cloning Kit, without competent cells

Product No. 450245, 11596885

Lot No. 2843290

Date of Manufacture 15-Feb-2024

### Restriction Enzyme Analysis

The parental supercoiled plasmid is qualified by restriction digest to confirm its identity prior to 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) |
|--------------------|-------------------------|
| <i>Mlu</i> I       | 2000 (sc)               |
| <i>Dra</i> I       | 3513 (linearize)        |
| <i>EcoR</i> V      | 3513 (linearize)        |
| <i>Pme</i> I       | 3120, 393               |
| <i>Pvu</i> II      | 2047, 593, 457, 360     |

**Results: Meets specification**

### TOPO Cloning

Each lot is qualified using control reagents included in the kit. Under conditions described in the accompanying manual, a 750-800 bp control PCR product is amplified and TOPO®-Cloned into pCR®-Blunt II TOPO® vector and subsequently transformed into One Shot® competent *E.coli* included with the kit. The following result must be obtained:

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

**Results: Meets specification**

### M13 (-20) Forward and M13 Reverse Primers

The M13 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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A handwritten signature in blue ink, appearing to read 'Chevohn Joseph', with a stylized flourish at the end.

Chevohn Joseph  
Director, Quality  
Issued on 16-Feb-2024