

Certificate of Analysis

pBAD202 Directional TOPO® Expression Kit

Product No. 450249
Lot No. 2269010
Date of Manufacture 19Mar2021

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.

<u>Restriction Enzyme</u>	<u>Expected Fragments (bp)</u>
<i>Bam</i> H I	443, 3995
<i>Eco</i> R I	4438 (linearize)
<i>Pme</i> I	4438 (linearize)
<i>Xmn</i> I	202, 4236
<i>Sac</i> I	4438 (linearize)
<i>Sph</i> I	1743, 2695

Note: The restriction sites used to qualify the parent vector may no longer be present in the topoisomerase I adapted vector.

Results: Meets specification

Directional TOPO Cloning

Each lot is qualified using control reagents included in the kit. Under conditions described in the accompanying manual, a 750 bp control PCR product is amplified using a forward primer containing CACC at its 5' end and a reverse primer. The product is TOPO® Cloned into pBAD202/D-TOPO® vector and subsequently transformed into One Shot® chemically competent *E. coli* included with the kit. The following results must be obtained:

- 1) $\geq 85\%$ Cloning Efficiency of pBAD202/D-TOPO® and directional PCR product ligation based on colony counts from plus insert plates and vector only plates.
- 2) $\geq 90\%$ (36 of 40) transformants analyzed from plus insert plates must contain the test PCR product cloned in the correct orientation as determined by Directional Colony PCR and/or restriction digest. Refer to manual for a list of restriction enzymes used to confirm orientation.
- 3) Relatively few colonies should be produced in the vector-only reaction.

Results: Meets specification

Sequencing Primers

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. If you have any further questions about this Certificate of Analysis, please contact Technical Services at 1-800-955-6288 (US and Canada) or 1-760-603-7200, x2 (all other countries).

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