

Datasheet for ABIN4710131

Human RPE65 cDNA Clone in Bacterial Expression Vector (His-MBP)

Overview

Quantity:	500 ng
Gene:	RPE65
Species:	Human
Fusion tag:	His-MBP
Insert:	cDNA
Vector:	Bacterial Expression Vector
Application:	Cloning (Clon)

Product Details

Purpose:	Bacterial expression of Human RPE65 with His-MBP
Insert Length:	1602 bp
Vector Backbone:	pPB-His-MBP
Promoter:	T7 Promoter
Bacterial Resistance:	Kanamycin
Expression Type:	Transient
Specificity:	5-NheI and 3-XhoI Fusion tag: Dual N-terminal tag, 6X Histidine followed by Maltose Binding Protein which is cleavable with Thrombin (Size 43 kDa)
Sequencing Primer:	MBP Forward primer: 5'-CGCAGATGTCCGCTTTCTGG-3', T7 terminator primer: 5'-GCTAGTTATTGCTCAGCGG-3'

Target Details

Gene:	RPE65
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Target Details

Alternative Name: RPE65 ([RPE65 Products](#))

NCBI Accession: [NM_000329](#)

Application Details

Application Notes: The pPB vectors are low-medium copy number vectors in which the gene expression is driven by the strong T7 promoter.

Below are some basic guidelines for using the pPB vectors for protein production:

1. The pPB vectors are designed to be used with E. coli strains that are DE3 lysogens i.e. the host E. coli cell has a source of T7 RNA polymerase.
2. Recombinant protein induction is usually done at OD600 of 0.6-1.2 using Isopropyl β -D-1-thiogalactopyranoside (IPTG) at a final concentration of 0.05 -1mM.
3. The ideal concentration of IPTG must be determined empirically for each recombinant protein/cell-line. Similarly, the length of time and temperature for induction provide other variables that need to be optimized on a case-to-case basis.
4. For toxic proteins, it is recommended to go for shorter induction time and also to try and suppress basal recombinant gene expression through (a) addition of glucose or use of pLysS plasmid. Please note that special cell-lines are also available in the market that cater to expression of toxic proteins.
5. Once grown for the desired length of time, harvest cells by centrifugation and either freeze the cells at -80°C (as such or after re-suspending in the desired buffer) or proceed with the purification.

Restrictions: For Research Use only

Handling

Format: Liquid

Buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

Storage: -20 °C

Storage Comment: 1 year when stored at -20° C or lower in a non-frost free freezer.

Expiry Date: 12 months

Publications

Product cited in: Johnson, Drugan, Miller, Evans: "38" in: , Vol. 1363, Issue Nucleic acids research, pp. 28-39, (

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