

pKYB1 Vector



1-800-632-7799
info@neb.com
www.neb.com



N6706S 002111114111

N6706S

10 µg Lot: **0021111** Exp: **11/14**
200 µg/ml Store at **-20°C**

Description: pKYB1 is an *E. coli* expression vector for use with the IMPACT™ Kit (1,2). It is designed for in-frame insertion of a target gene into the polylinker, upstream of the Sce VMA intein/chitin binding domain (55 kDa)(1,3). pKYB1 carries the kanamycin resistance gene (Kn) from Tn 903. This double stranded vector is 8,393 bp in length.

Source: pKYB1 is isolated from an *E. coli* strain (r-m-) by a standard plasmid purification procedure.

Supplied in: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

Features of this vector:

- A pBR322 derivative
- The NdeI site in the polylinker contains an ATG sequence for translation initiation.
- Use of the SapI site allows for cloning of the target protein adjacent to the intein, resulting in purification of the target protein without any additional amino acids at its C-terminus.

Polylinker Region:

```
pKYB1
5'...CGG GGA TCT CGA TCC CGC GAA ATT AAT ACG ACT CAC TAT AGG GGA ATT GTG AGC
                                     T7 Promoter          lac operator
GGA TAA CAA TTC CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA
                                     XbaI                      Shine Dalgarno
Met Ala Ser Ser Arg Val Asp Gly Gly Arg Glu Phe Leu Glu Gly Ser Ser Cys1
CAT ATG GCT AGC TCG CGA GTC GAC GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC
NdeI NheI NruI Sall NotI EcoRI XhoI SapI
                                     Intein →
TTT GCC AAG GGT ACC AAT GTT TTA ATG GCG GAT GGG TCT ATT GAA TGT ATT
                                     KpnI
GAA AAC ATT GAG GTT GGT AAT AAG GTC ATG GGT ...3'
← Intein Reverse Primer
```

- Unique sites are in bold. NheI and NruI are not unique.
- Expression of the fusion gene is under the stringent control of the T7 promoter (4) and is regulated by IPTG due to the presence of a *lac I* gene.
- Origin of DNA replication from bacteriophage M13, which allows for the production of single-stranded DNA by helper phage superinfection of cells bearing the plasmid (M13K07 Helper Phage, NEB #N0315)

- Kanamycin resistant
- Compatible restriction sites for subcloning a fusion gene from other IMPACT vectors.
- A wide range of *E. coli* host strains: T7 Express Competent *E. coli* (High Efficiency) (NEB #C2566) or BL21(DE3) Competent *E. coli* (NEB #C2527) and derivatives.

References:

1. Chong, S., Mersha, F.B., Comb, D.G., Scott, M.E., Landry, D., Vence, L.M., Perler, F.B., Benner, J., Kucera, R.B., Hirvonen, C.A., Pelletier, J.J., Paulus, H. and Xu, M. -Q. (1997). Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element. *Gene* 192, 271–281.
2. Chong, S., Shao, Y., Paulus, H. Benner, J., Perler F.B. and Xu, M. -Q.(1996). Protein splicing involving the *Saccharomyces cerevisiae* VMA intein: the steps in the splicing pathway, side reactions leading to protein cleavage, and establishment of an *in vitro* splicing system *J. Biol. Chem.* 271, 22159–22168.

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CERTIFICATE OF ANALYSIS

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                                     XbaI                      Shine Dalgarno
Met Ala Ser Ser Arg Val Asp Gly Gly Arg Glu Phe Leu Glu Gly Ser Ser Cys1
CAT ATG GCT AGC TCG CGA GTC GAC GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC
NdeI NheI NruI Sall NotI EcoRI XhoI SapI
                                     Intein →
TTT GCC AAG GGT ACC AAT GTT TTA ATG GCG GAT GGG TCT ATT GAA TGT ATT
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CERTIFICATE OF ANALYSIS

3. Watanabe, T., Ito, Y., Yamada, T., Hashimoto, M., Sekine, S. and Tanaka, H. (1994). The role of the C-terminal domain and type III domains of chitinase A1 from *Bacillus circulans* WL-12 in chitin degradation. *J. Bacteriol.* 176, 4465–4472.
4. Dubendorff, J.W. and Studier, F.W. (1991). Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with *lac* repressor *J. Mol. Biol.* 219, 45–59.

Additional information such as vector sequences and frequently asked questions, are available at www.neb.com.

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3. Watanabe, T., Ito, Y., Yamada, T., Hashimoto, M., Sekine, S. and Tanaka, H. (1994). The role of the C-terminal domain and type III domains of chitinase A1 from *Bacillus circulans* WL-12 in chitin degradation. *J. Bacteriol.* 176, 4465–4472.
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U.S. Patent Nos. 5,496,714, 5,834,247

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