pMYB5 Control Plasmid

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info@neb.com
www.neb.com

N6906S 004100413040

N6906S

10 µg **Lot: 0041004** **Exp: 4/13**

0.20 mg/ml **Store at –20°C**

Description: pMYB5 Control Plasmid is a control plasmid for the IMPACT™ Kit (1,2). This plasmid carries the *E. coli malE* gene, encoding the maltose binding protein (MBP)(3), fused in-frame to the coding region of the *Sce* VMA intein-chitin binding domain (55 kDa)(1,4). pMYB5 can be used to test plasmid transformation, cell culture, induction and purification procedures. After induction with 0.3 mM IPTG at 30°C for 3 hours (or 15°C for 12–16 hours), 100 ml of cells should yield 2–3 mg of a 97 kDa fusion protein. After chitin column purification and cleavage, approximately 1.0–1.5 mg of the MBP (42 kDa) is usually obtained. This double stranded vector is 8602 bp in length.

Source: pMYB5 Control Plasmid is isolated from an *E. coli* strain (r- m-) by a standard plasmid purification procedure.

Supplied in: 10 mM Tris-HCl (pH 8.0 @ 25°C), 1 mM EDTA.

Features of pMYB5 Control Plasmid:

- Expression of the fusion gene is under the control of an IPTG -inducible T7 promoter (5).
- *E. coli* strains T7 Express Competent *E. coli* (High Efficiency) (NEB #C2566) or BL21(DE3) Competent *E. coli* (NEB #C2527) and derivatives can be used as expression hosts.
- A pTYB1 derivative
- Ampicillin resistance

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.
3. Guan, C.D. Li. P., Riggs, P.D., and Inouye, H. (1988). Vectors that facilitate the expression and purification of foreign peptides in *Escherichia coli* by fusion to maltose-binding protein Gene 67, 21–30.
4. 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.
5. 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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U.S. Patent Nos. 5,496,714, 5,834,247

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