

## Dimethylsulfoxide and Sodium Butyrate Enhance the Production of Recombinant Cyclooxygenase 2 in Stably Transformed *Drosophila melanogaster* S2 Cells

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### Objectives

The purpose of this experiment is to optimize the yield of the recombinant Cox2 from the stably transformed *Drosophila melanogaster* S2 cells, using dimethylsulfoxide and sodium butyrate.

### Materials and Methods

Materials - Cell line : *Drosophila melanogaster* Schneider 2 (S2) cells

- vector : pMT/BiP/V5-His and pCoHygro (Invitrogen)

Methods - Construction of expression plasmids, Stable transformation,

Cell culture and analysis of gene expression, Purification of recombinant Cox 2,

Protein determination and Cox 2 assay, SDS-PAGE and Western blot analysis

### Results and Discussion

Recombinant human cyclooxygenase 2 (Cox 2) was expressed in stably transformed *Drosophila melanogaster* S2 cells, and was present primarily in the cellular fraction at a molecular weight of 70 to 74 kDa. Recombinant Cox 2 was purified using Ni<sup>2+</sup>-affinity fractionation, and its specific activity was 24,800 Unit mg<sup>-1</sup>. The peak level of recombinant Cox 2 production was 1.6 µg (10<sup>7</sup> cells)<sup>-1</sup>, seven days after induction with 0.5 mM CuSO<sub>4</sub>. Supplementing the cultures with dimethylsulfoxide or sodium butyrate increased recombinant Cox 2 production by 170% and 86%, respectively.

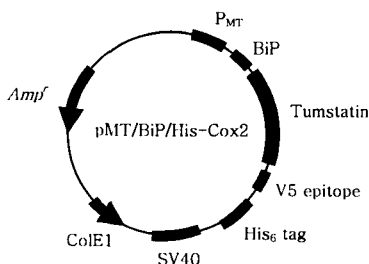


Fig.1. Schematic representation of the expression plasmid, pMT/BiP/His-Cox2.

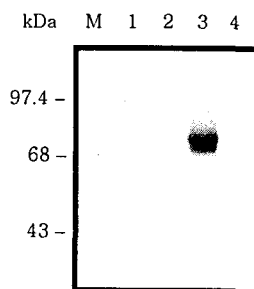


Fig. 2. SDS-PAGE (A) and Western blot analysis (B) of the purification of recombinant Cox 2 from stably transformed S2 cells.

## **References**

- Arts J, Lansink M, Grimbergen J, Toet KH, Kooistra T (1993) Stimulation of tissue plasminogen activator gene expression by sodium butyrate and trichostatin A in human endothelial cells involves histone acetylation. *Biochem. J.* **310**, 171-176.
- Campbell PL, Kulozik AE, Woodham JP, Jones RW (1990) Induction by HMBA and DMSO of genes introduced into mouse erythroleukemia and other cell lines by transient transfection. *Genes Dev.* **4**(7), 1252-1266.
- Cromlish WA, Payette P, Culp SA, Quellet M, Percival MD, Kennedy BP (1994) High level expression of active human cyclooxygenase-2 in insect cells. *Arch. Biochem. Biophys.* **314**, 193-199.
- George HJ, Van Dyk DE, Straney RA, Trzaskos JM, Copeland RA (1996) Expression, purification and characterization of recombinant human inducible prostaglandin G/H synthase from baculovirus-infected insect cells. *Protein Expr. Purif.* **7**, 19-26.
- Herschmann H (1995) Prostaglandin synthase 2. *Biochim. Biophys. Acta* **1299**, 125-140.
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of head of bacteriophage. *Nature* **227**, 680-685.
- Miller DB, Munster D, Wasvary JS, Simke JP, Peppard JV, Bowen BR, Marshall PJ (1994) The heterologous expression and characterization of human prostaglandin G/H synthase-2 (Cox 2). *Biochem. Biophys. Res. Commun.* **201**, 356-362.
- Oh SKW, Vig P, Chua F, Teo WK, Yap MGS (1993) Substantial overproduction of antibodies by applying osmotic pressure and sodium butyrate. *Biotechnol. Bioeng.* **42**, 601-610.
- Oster T, Thioudellet C, Chevalot I, Masson C, Wellman M, Marc A, Siest G (1993) Induction of recombinant human gamma-glutamyl transferase by sodium butyrate in transfected V79 and CHO Chinese hamster cells. *Biochem. Biophys. Res. Commun.* **193**, 406-412.
- Palermo DP, DeGraaf ME, Marotti KR, Rehberg E, Post LE (1991) Production of analytical quantities of recombinant proteins in Chinese hamster ovary cells using sodium butyrate to elevate gene expression. *J. Biotechnol.* **19**, 35-48.