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SIMULTANEOUS EXPRESSION OF HUMAN CYTOCHROME P450 3A5 AND NADPH-CYTOCHROME P450 REDUCTASE IN CHINESE HAMSTER OVARY CELL USING INTERNAL RIBOSOME ENTRY SITE

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For a continuous expression of human cytochrome p450 3A5 (CYP3A5) and NADPH-cytochrome P450 reductase (CYPR) proteins, bicistronic construct (CYP3A5BC-LNCX2) was made using internal ribosome entry site (IRES). As for mammalian cell expression, we used pLNCX2 retroviral vector; and using calcium phosphate, plasmid transfer was achieved in 293GPG cell and transduced in Chinese hamster ovary (CHO) cell. Using neomycin and limiting dilution method, the CHO cells for protein expression were established (CHO.3A5BC-LNCX2); and after culturing the host cells, Western blotting were carried out on the microsomes obtained after the process with sonication and ultracentrifugation. The molecular weight of CYP3A5 was determined to be 57kDa and that of CYPR, 76kDa, respectively. The results of MTS assay to measure the cytotoxicity of 2-aminoanthracene (2-AA), 2-amono-3,4-dimethylimidazo[4,5-*f*]quinoxaline (MelQx), aflatoxin B_1 and aflatoxin B_2 on CHO.3A5BC-LNCX2 showed that the sensitivity rate was 50 folds in 2AA and more than two folds in other materials compared to the negative control.