www.biochemj.org

Supplement for EXPERIMENTAL in BJ2006/1118

Construction of expression vectors

The expression vector pcDNA3.1(+)/ST6GalNAc III was prepared by inserting a HindIII and XhoI fragment from pT-Adv vector/ST6GalNAc III into the HindIII and XhoI sites of pcDNA3.1(+) vector. The expression vector pcDNA3.1-hST6GalNAc V was prepared by inserting an EcoRI and SpeI fragment from pME18SFL3-hST6GalNAc V (purchased from Helix Research Institute, Chiba, Japan) into the EcoRI and Spe I sites of pcDNA3.1 (+) vector. The expression vector pcDNA3.1-hST6GalNAc VI was prepared by inserting an EcoRV and Hind III fragment from pCR®2.1-hST6GalNAc VI (provided by A. Yoshida at Central Laboratories for Key Technology, Kirin Brewery Company, Yokohama, Japan) into the EcoRV and Hind III sites of pcDNA3.1 (-) vector.

Northern blotting

The expression levels of the hST6GalNAc III and VI genes in human renal cancer cell lines and normal human renal proximal tubule epithelial cells were determined by Northern blotting. Total RNA was prepared using TRIZOL Reagent™ (Invitrogen) according to the manufacturer's instructions. Fifteen micrograms each of total RNA were electrophoresed and blotted onto a GeneScreen Plus® membrane (PerkinElmer Life Sciences). Hybridization was carried out for 16 h at 42°C with [^{32}P] dCTP-labeled hST6GalNAc III or VI full-length cDNA probes. The membranes were washed, then exposed to the imaging plate to be visualized with a BAS 2000 image analyzer (Fuji

Film, Tokyo, Japan). Expression levels of hST6GalNAc III or VI mRNA were assessed as the ratio to those of β-actin mRNA using the NIH Image program. The expression levels of the hST6GalNAc III and VI genes in various human tissues were also determined as described above using Human Northern RNA blot-12 major Tissues™ (OriGene Technologies, MD).