

HEPAC²: Serum-free, long-term, re-usable human hepatocyte cultures

PRIMACYT has developed HEPAC², a standardized and validated serum-free, long-term and re-usable human hepatocyte culture system. Human hepatocytes are cultured on collagen-coated plates in the presence of HHMM (Human Hepatocyte Maintenance Medium). Hepatocyte specific functions like ammonia detoxification and blood synthesis are maintained for several weeks. Cytochrome P450 associated enzymatic activities can be induced several times within one hepatocyte culture (Fig. 1).

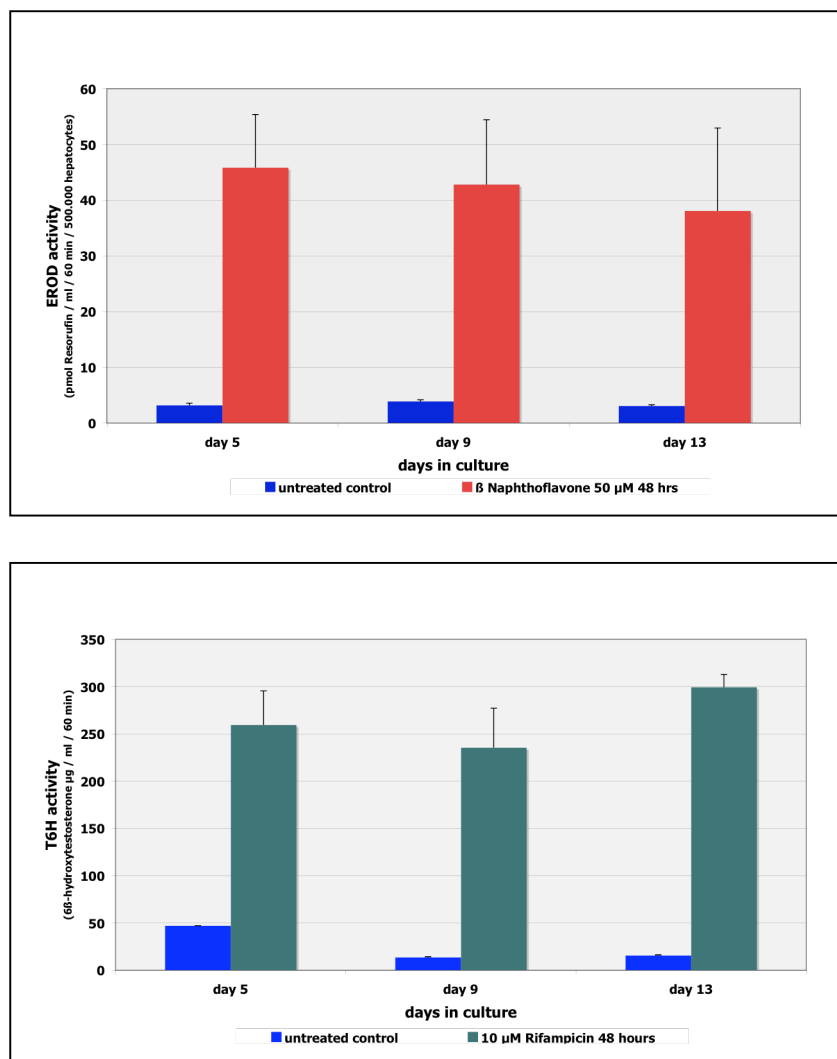


Fig. 1: Cytochrome P4501A2 associated Ethoxyresorufin-O-deethylase (EROD, upper panel) and cytochrome P4503A4 associated Testosterone-6 β -hydroxylase (T6H, lower panel) activity can be induced repeatedly in human hepatocytes cultured with HHMM.

We have implemented a standard protocol that allows the repeated use of human hepatocytes for several cycles of drug analyses, as long the substance(s) of interest or its metabolites are non toxic (Figs. 2 and 3).

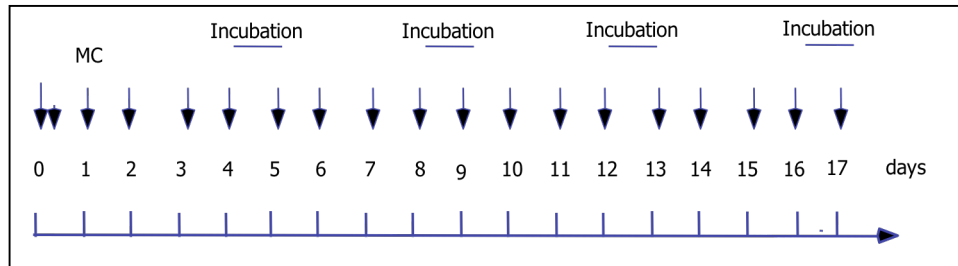


Fig. 2: Implementation of a „recyclable“ human hepatocyte culture system. MC, arrows = medium change. Human hepatocytes are cultured for 4 days with daily medium changes. On day 4, the first incubation cycle is started and hepatocytes are incubated with the substance(s) of interest for up to 24 hours. After 24 hours the medium is changed and the cells are cultured in the absence of assay substance(s) for 72 hours. The incubation cycle is repeated every 4 days on day 8, 12, 16 etc.

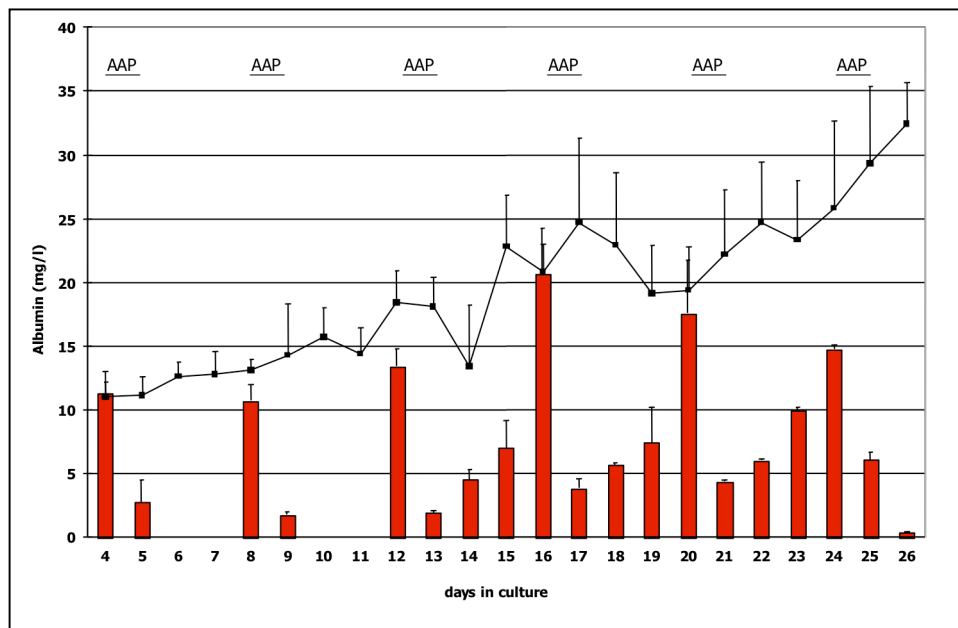


Fig. 3: Use of acetaminophen (AAP) as a model substance to establish HEPAC² as a re-usable hepatocyte culture system. Human hepatocytes were cultured in HHMM. Medium was changed every 24 hours. Albumin was detected in cell culture medium (control, black line). At days indicated, acetaminophen was added for 24 hours. Albumin release before, during and after incubation with AAP was determined in the culture medium (red bars). During each incubation cycles AAP reduced albumin release by 70-80%. This effect was reversible, within 3 days albumin release returned to normal control levels. The incubation cycle could be performed 5 times, each time AAP reduced albumin release by about 75 %. Data are from Ullrich et al. (ALTEX 24:35-40, 2007).