ALB secretion ability with time, the culture supernatants were sampled every two days for the first 6 days of hepatocyte induction. In order to determine the ALB secretion ability after the completion of hepatocyte induction, the overlaying Matrigel was suctioned to be removed on Day 8. Half of these cells were collected for measuring the DNA content while the other half were added with a fresh medium to culture for another 2 days. On Day 10, the culture supernatant was corrected to measure the ALB secreted during Day 8 to 10, which was normalized with the DNA content on Day 8. The DNA content was measured using DNA Quantity Kit (Cosmo Bio, Tokyo).

[0051] Determination of CYP1A activity

In order to induce CYP1A activity, cells on Day 8 of hepatocyte induction were treated with 5 μM 3-methylcholanthrene (3-MC) (Sigma) for 4 days (the medium was exchanged on the second day). The control cells were treated only with DMSO as a solvent. Four days later, P450-Glo CYP1A1 Assay (Luciferin-CEE) was used to determine CYP1A activity. According to the manufacturer's instruction, this kit can detect CYP1A2 activity more efficiently than CYP1A1 activity. Following CYP1A activity, the DNA content of the cells was measured to normalize the CYP activity. In order to examine the responsiveness to 3-MC, fold changes in activity were calculated as [mean luminescence in the presence of 3-MC/mean luminescence in the absence of 3-MC] for 3 wells/condition in each experiment to determine an average value for five independent experiments. In two experiments, total RNA was isolated to assess the gene expression levels of CYP1A1 and CTP1A2.

[0052] Fluorescein diacetate assay

For hepatocyte induction, the cells were incubated in a medium containing 2.5 μg/mL fluorescein diacetate (FD) (Sigma) in a CO_2 incubator for 15 minutes. After replacing the medium with Hanks' balanced salt solution (HBSS) (Life Technologies), metabolized fluorescein was detected under a fluorescence microscope. For bile duct induction, following 15 minutes of incubation, the medium was freshly exchanged, and culture was continued for another 30 minutes to induce transportation of metabolized fluorescein to the lumen. Then, the medium was replaced with HBSS to observe fluorescein distribution under a fluorescence microscope.