121

Colorimetric Assay System for Screening Antiviral Compounds against Hepatitis B Virus.

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A highly sensitive, rapid, and accurate assay system was developed for the in vitro evaluation of anti-hepatitis B virus (anti-HBV) agents. Chronic HBV-like particle producing HB611 cells were used in combination with immunoaffinity purification, polymerase chain reaction (PCR), and hybrid capture detection. HB611 cells were incubated with putative anti-HBV agents for 7 days in 96-well microtiter plates. Using the MTT method, the cytotoxic effects of compounds were assayed in parallel with antiviral activity. HBV-like particle was purified from HB611 cell culture media using immunoaffinity purification. The HBV DNA was extracted, amplified with PCR, and assayed using a hybrid capture colorimetric method. This assay provided quantitative detection of extracellular HBV DNA from cell culture media. We found that 50% effective concentration levels of several known anti-HBV agents (HPMPA, PMEDAP , PMEA and others) obtained by the colorimetric method were similar to those reported in studies using Southern blot analysis. These results demonstrate that this simple and easily automated colorimetric assay system is useful for assessment of anti-HBV compounds.

122

Novel Assay System for Hepatitis C Virus Serine Protease Inhibitors

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Novel assay system was developed for in vitro evaluation of hepatitis C virus (HCV) protease (Cpro2) inhibitor with transient expression system using COS-1 cells.
The HCV protease assay system is based on expression of a luciferase gene transactivated by HTLV-1 tax. Tax protein is designed to release from cellular membrane and to translocate into the nuclei when active HCV serine protease is produced. Twenty hours after the co-transfection, the culture medium was changed to the fresh medium and then the cells were cultured for another 20h. COS-1 cells were lysed with the lysis buffer and luciferase activity was measured in a 96well micro- plate by a luminometer. The luciferase activity was only detected in the cells co-transfected with complete sets of plasmids. In this system we could measure specifically the HCV protease activity as the luciferase activity. This assay system is useful for evaluation of compounds for their inhibitory effect on HCV proteinase activity as well as cytotoxicity to cells.