

Hep3B cell line (ATCC HB-8064) was maintained in EMEM with 10% FBS at 37 °C in a humidified atmosphere (air supplemented with 5% CO₂) and passaged with 0.05% trypsin at a subcultivation ratio of 1:3. 1×10^6 Hep3B cells were mixed with siRNA loaded in SP94-targeted DOPC protocells such that the final siRNA concentration was 125 pM. Loaded siRNA silenced expression of cyclin A2. After incubation at 37 °C for various periods of time cells were washed three times with cold 1 x PBS to remove excess protocells. mRNA was then isolated from cells and converted to cDNA using the TaqMan Fast Cells-to-CT kit. Quantitative PCR was performed by SeqWright, Inc. (Houston, TX, USA).