Optimizing Medium for Transient Transfection

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Abstract: During the development of a transient transfection platform, it was observed that several commonly used components were able to support high transfection efficiency and protein production when added at certain steps of the transfection process. Transfection could be inhibited or completely diminished if such critical components were present at other steps of the process. Our data demonstrated that a transfection protocol can be as critical to efficient polyethylenimine (PEI) mediated transient transfection of human embryonic kidney (HEK) 293 EBNA cells as the transfection medium.

Key words: Serum-Free medium, process development, PEI, HEK.293 EBNA, transient transfection

1. INTRODUCTION

Because components required for cell growth and protein production can inhibit or diminish transfection efficiency when present in transfection medium, development of one medium for the entire transient transfection process can be extremely difficult. A protocol that utilizes different media at different stages of the transfection process not only improves the transfection efficiency but also the viable cell density and total protein production.

2. MATERIALS AND METHODS

EX-CELL 60864 (JRH Biosciences, Inc.) cultured HEK 293 EBNA (CRL-10852, ATCC) cells were diluted at log phase growth with variations

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of transfection media (EX-CELL™ 293, EX-CELL 60864 and EX-CELL 65237) at a 1:4 ratio to reach a cell density of (4-6)e^5/mL. Cells were seeded at 1mL/well in a 12-well non-tissue culture treated plate (Catalog No. 351143, Falcon, Bedford, MA). Two hours later DNA and PEI complex were added to the cell culture.

To make the DNA (P040400, Gene Therapy Systems Inc) PEI (23966, Polysciences, Inc)complex for 1mL of cell suspension, 3.6ug of PEI was added to 200uL of transfection medium, vortex for 30 seconds, 2.4ug of DNA was then added in the mixture. After 30 minutes, the DNA/PEI complex was added to the cell suspensions.

The level of GFP was quantitated after 72 hours post transfection by fluorescence microscopy using a Spectra MAX Gemini XS plate reader (λex=480 nm λem=510 nm).

3. RESULTS

The data from Figure 1 showed that the formulations containing either dextran sulfate, a high concentration of hydrolysate or a high concentration of phosphate, yielded zero or significantly reduced transfection efficiencies.

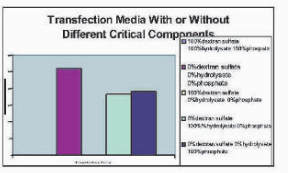

Figure 1. GFP fluorescence intensity comparison for different transfection media.

Without these components in the medium viable cell density was decreased (Figures 2 and 3).

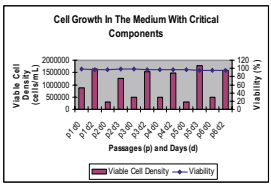

Figure 2. Cell growth in complete media.

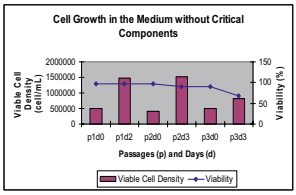

Figure 3. Cell growth in deficient medium.

Feeding the cells with complete medium containing dextran sulfate, hydrolysate and phosphate 16 hours after transfection improved total protein productivity as shown in Figure 4.

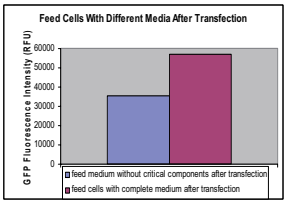

Figure 4. GFP fluorescence intensity for the transfected GFP cells with different media.

4. CONCLUSION

Dextran sulfate has the most significant negative effect on transfection efficiency. It completely inhibited transfection. At 100% concentration hydrolysate and phosphate also demonstrated significant negative effects on transfection efficiency by causing 20% reduction in efficiencies. However, by performing the transfection process in a deficient medium, followed by feeding cells with a complete medium 16 hours after transfection, 50% transfection efficiency were achieved in combination with good viable cell densities, viabilities and protein production.