ABSTRACT

In recent years, the human cell line HEK293 has become a key tool for recombinant protein expression, particularly in the context of gene therapy. Understanding the conditions that ensure efficient and cost-effective expression of recombinant proteins is crucial. This study focused on optimizing transient transfection in human cell lines HEK293T and MEXi-293E, with the main goal of enhancing the efficiency of this method as well as ensuring effective protein yield. To determine these conditions, easily detectable reporter proteins – green fluorescent protein (GFP) and secreted alkaline phosphatase (SEAP) – were used. The optimization process involved comparing three types of transfection reagent linear polyethyleneimine, its ratios to DNA, and the conditions, under which the transfection occurred. Transfection efficiency was determined by the fluorescence signal of GFP using flow cytometry, while production efficiency was measured by spectrophotometry based on the catalytic activity of SEAP.

KEY WORDS

HEK293, recombinant expression, transfection, tissue culture, flow cytometry