HEK293 is a human cell line derived from embryonic kidney cells and is a frequently used system for the production of recombinant proteins. This work dealt with optimization of the composition of serum-free medium for HEK293S and HEK293T cell lines as a compensation for expensive commercial media. The growth of culture and expression of reporter proteins SEAP and GFP was monitored as the markers. I managed to create a new medium which contained, among other compounds, insulin (1 mg/l), transferrin (5 mg/l) and a mixture of trace elements. During the cultivation in a mixture of commercial medium EX CELL 293 with my new medium 293S cells grew faster than during the cultivation in commercial media (doubling time 20,47 ± 2,68 hours (srel = 13,1 %)). It seems that the new medium is suitable for transfection of HEK293 cell lines with a relatively high expression of recombinant proteins. Transfection ratio of DNA:PEI (w/w) for this medium is 1:2 to 1:3.