HYPE-5™ Transfection Kit Results

HYPE-5™ Transfection Kit is dedicated to achieve **H**igh **Y**ield **P**rotein **E**xpression in mammalian cells. This Kit has been designed for maximum efficiency in HEK293 and CHO cells growing in suspension. The transient transfection expression system represents an ideal and faster alternative to the stable expression system (costly and time-consuming). Scale-up to larger volumes for production of milligrams of protein per liter of cell culture is straightforward and easy with simple and cost efficient handling steps.

Main features are:

1. High protein production yield.
2. Suitable for both HEK293 and CHO cells growing in suspension.
3. Compatible with any synthetic or regular media used for protein production.
4. Ideal for bioreactor, spinner or flasks.
5. Rapid, simple to scale up and ready-to-use.
6. Free from animal sources.

Applications

HYPE-5™ Transfection Kit has been designed for large scale up transient transfection and high protein expression. The system is optimized for maximum efficiency in HEK293 and CHO cells. It has been used and validated with cells from different origins (FreeStyle™ CHO-S/293-F cells from Invitrogen, CHO protein free from ECACC, etc.) cultured in suspension (flask, spinner and bioreactor).

HYPE-5™ Kit is suitable for any synthetic or regular culture media. It has been tested with various chemically defined media:

Medium	HEK293	CHO
FreeStyle™ CHO-S	NA	✓
FreeStyle™ 293	✓	NA
ProCHO™ 4-5	NA	✓
Pro293™S-CDM	✓	NA
EX-CELL® ACF CHO	NA	✓
EX-CELL® 293	✓	NA
BD Select™ CD 1000	NA	✓

NA: Not Applicable

The results shown below have been obtained with FreeStyle™ 293/CHO-S systems (Invitrogen) and the maximum efficiency has been reached when HYPE-5 reagent was used alone for HEK293 cells or combined with HYPE-Blast for CHO cells.

Efficient protein production is highly dependent on the cell model used. For instance, adaptation of cells to growth in suspension, culture medium and cell density parameters are of importance to obtain the maximum efficiency. We advise optimizing experimental conditions (see instruction manual, section 4) in order to achieve the best efficiency.

High production yield for secreted protein

The results presented below indicate that HYPE-5™ Transfection Kit achieves the highest protein yield in comparison to other commercially available transfection reagents in HEK293 and CHO cells adapted in suspension with chemically defined medium and in absence of serum.

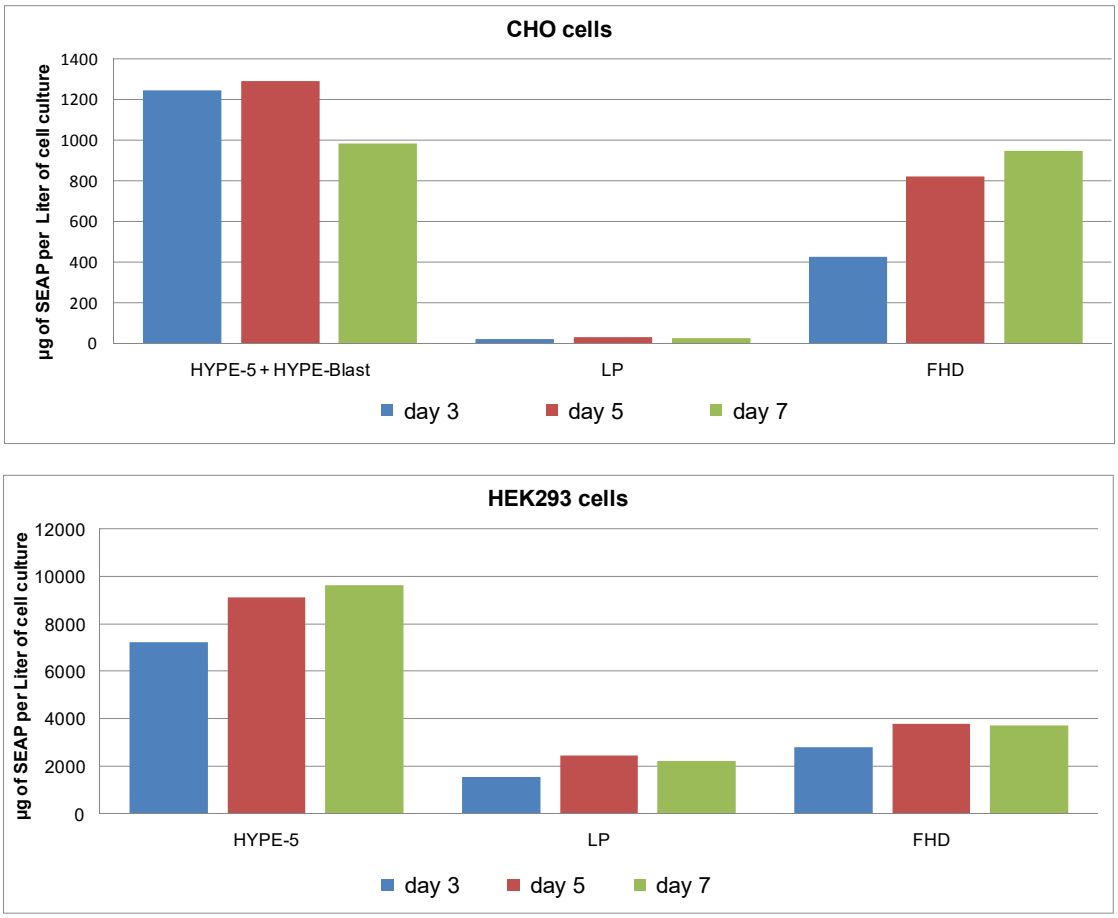


Fig.1: **SEAP Expression with HYPE-5 Kit vs. other reagents.**
Secreted Alkaline Phosphatase (SEAP) was produced by transient transfection using HYPE-5 Transfection Kit, LP or FHD transfection reagents according to manufacturer's instructions. Transfections were performed using 1.5 µg of plasmid DNA per mL of cell culture. Ratio (µL reagent per µg of plasmid) for HYPE-5 reagent, LP and FHD was 2, 2.5, and 3.5, respectively. CHO and HEK293 cells were cultured in 0.5 mL in a 24-well shaken plate. Density of cell culture was 1 x 10^6 cells/mL the day of transfection. 4 hours after transfection, for CHO cells only, HYPE-Blast was added to cell suspension. Day 3, 5 and 7, 20µL of supernatants were collected and SEAP activity was measured.

Outperforms competitor

The results presented below indicate that HYPE-5™ Kit outperforms other commercially available reagents specific for biomanufacturing in HEK293 and CHO cells adapted in suspension with chemically defined medium and in absence of serum.

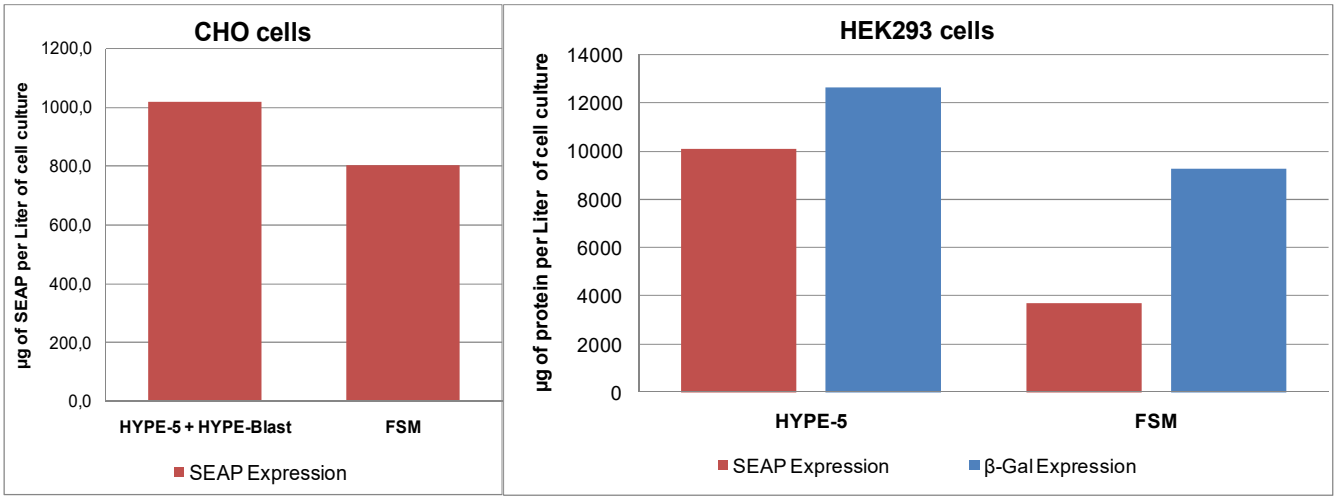
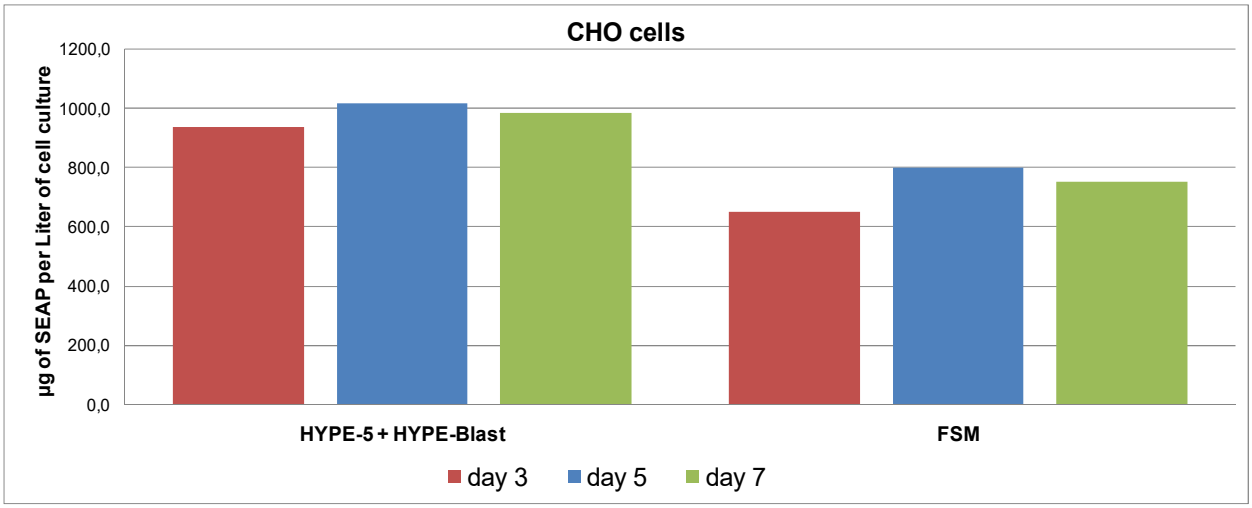


Fig.2: **SEAP/β-Gal Expression with HYPE-5 Kit vs. FSM reagent**.
Secreted Alkaline Phosphatase (SEAP) or β-Galactosidase (β-Gal) were produced by transient transfection using HYPE-5 Kit or FSM transfection reagents according to manufacturer's instructions. Transfections were performed using 1.5 µg of plasmid DNA per mL of cell culture. Ratio (µL reagent per µg of plasmid) for HYPE-5 reagent and FSM reagents was 2 and 1, respectively. CHO and HEK293 cells were cultured in 30 mL in 125 mL shaken flasks. Density of cell culture was 1 x 10^6 cells/mL the day of transfection. 4 hours after transfection, for CHO cells only, HYPE-Blast was added to cell suspension. Day 5 (CHO cells) and 7 (HEK293 cells) 500µL of supernatants were collected and SEAP or β-Gal activity was measured.



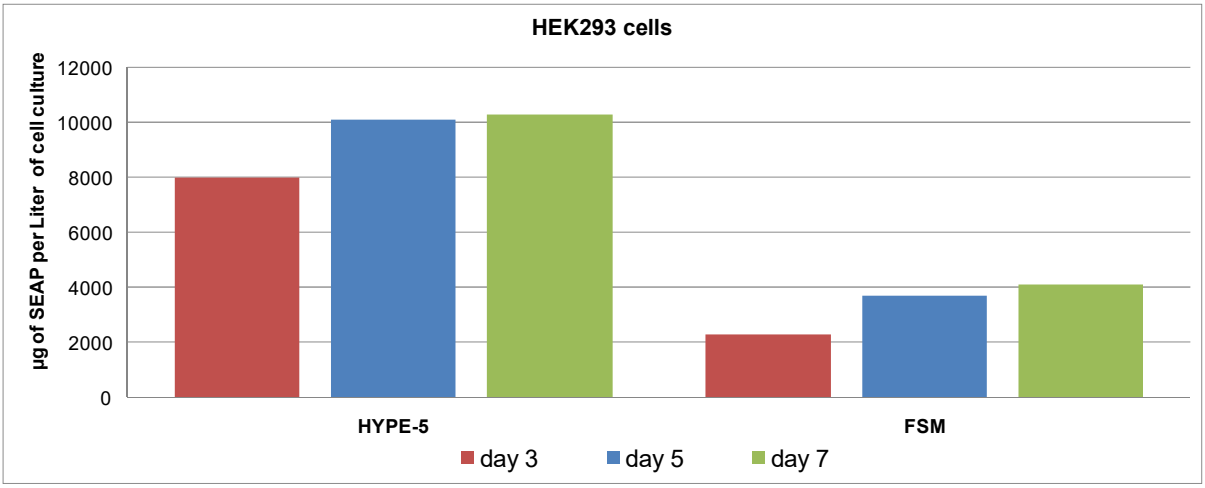


Fig.3: **SEAP Kinetic expression on cells transfected with HYPE-5 Kit vs. FSM reagent**.
Secreted Alkaline Phosphate (SEAP) was produced by transient transfection using HYPE-5 Kit or FSM transfection reagents according to manufacturer's instructions. Transfections were performed using 1.5 µg of plasmid DNA per mL of cell culture. Ratio (µL reagent per µg of plasmid) for HYPE-5 reagent and FSM reagents was 2 and 1, respectively. CHO and HEK293 cells were cultured in 30 mL in 125 mL shaken flasks. Density of cell culture was 1 x 10^6 cells/mL the day of transfection. 4 hours after transfection, for CHO cells only, HYPE-Blast was added to cell suspension. Day 3, 5 and 7, 500µL of supernatants were collected and SEAP activity was measured.

Easy and efficient scale-up

The results presented below indicate that protein production in CHO or HEK293 suspension cells can be easily scaled up by using HYPE-5™ Transfection Kit. HYPE-5™ Kit has been used in volume up to 300 mL of cell culture without any protocol modification and protein yield production is even higher when compared to lower cell culture volume.

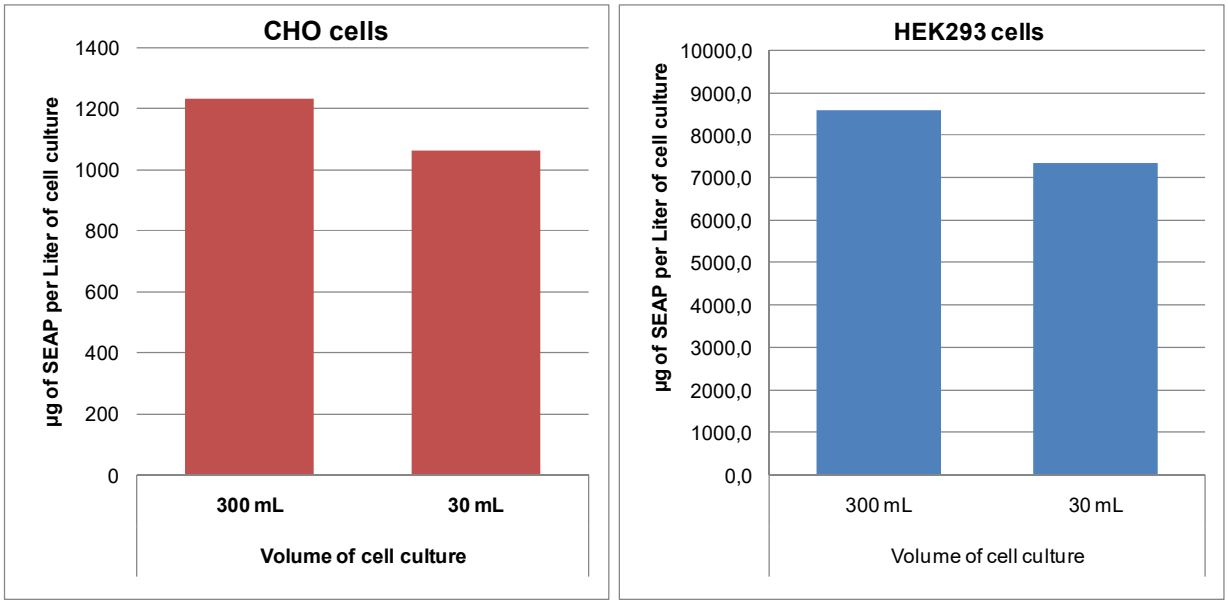


Fig.4: **SEAP Expression with HYPE-5 Kit in increasing volume of culture.**
Secreted Alkaline Phosphatase (SEAP) was produced by transient transfection using HYPE-5 Transfection Kit according to the instruction manual. Transfections were performed using 1.5 µg of plasmid DNA per mL of cell culture. Ratio of 2 µL of HYPE-5 reagent per µg of plasmid was used. CHO and HEK293 cells were cultured in 30 mL or 300mL of medium in 125 mL or 1000 mL shaken flasks, respectively. Density of cell culture was 1 x 10^6 cells/mL the day of transfection. 4 hours after transfection, for CHO cells only, HYPE-Blast was added to cell suspension. Day 5 (CHO cells) and 7 (HEK293 cells) 500µL of supernatants were collected and SEAP activity was measured.

High production yield for intracellular expressed protein

HYPE-5™ Kit is also highly efficient for intracellular protein production on cells adapted in suspension with chemically defined medium and in absence of serum.

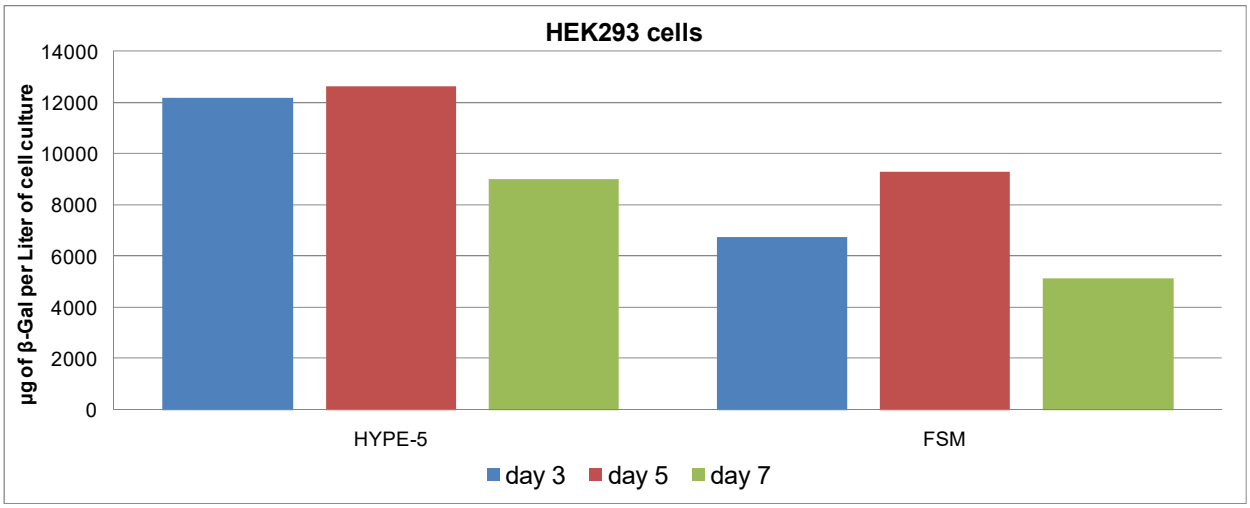
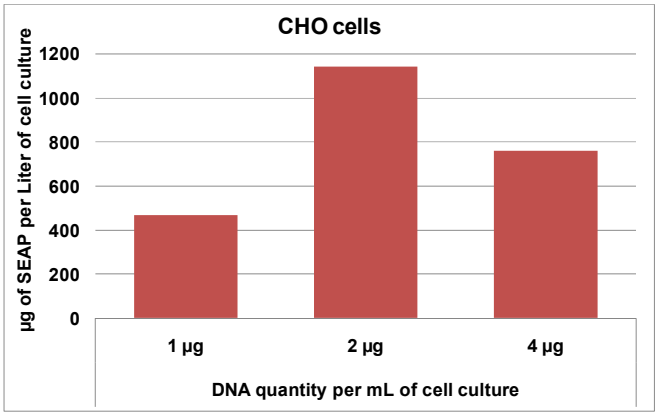


Fig.5: **β-Galactosidase Kinetic expression with HYPE-5 Kit vs. FSM reagent**.
β-Galactosidase was produced by transient transfection using HYPE-5 reagent or FSM transfection reagents according to manufacturer's instruction. Transfections were performed using 1.5 µg of plasmid DNA per mL of cell culture. Ratio (µL reagent per µg of plasmid) for HYPE-5 reagent and FSM reagents was 2 and 1, respectively. HEK293 cells were cultured in 30 mL in 125 mL shaken flasks. Density of cell culture was 1 x 10^6 cells/mL the day of transfection. Day 3, 5 and 7, 500µL of supernatants were collected and β-galactosidase expression was measured with ONPG kit (cat # GO10001).

Parameters influencing the protein production yield

Efficient protein production is extremely dependent on the cell model used. For instance, adaptations of cells to growth in suspension, culture medium and cell density (before and during transfection) are of importance to obtain the maximum efficiency. HYPE-5™ Transfection Kit has been used and validated with different cell origins and cultivated in different chemically defined medium. Other critical parameters are also important for efficient protein production. The results presented below show the influence of cell density and DNA quantity on protein production efficiency.

Fig.6-8: SEAP was produced by transient transfection using HYPE-5 Transfection Kit according to the instruction manual. CHO/HEK293 cells were cultured in 0.5 mL of medium in a 24-well shaken plate. 4 hours after transfection, for CHO cells only, HYPE-Blast was added to cell suspension. Day 5 (CHO) or 7 (HEK293), 20µL of supernatants were collected and SEAP activity was measured.



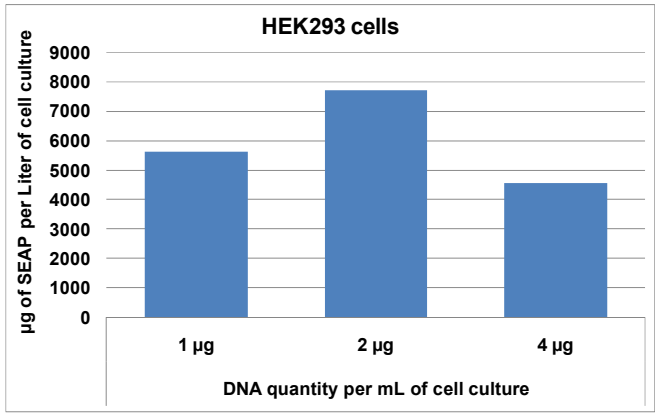
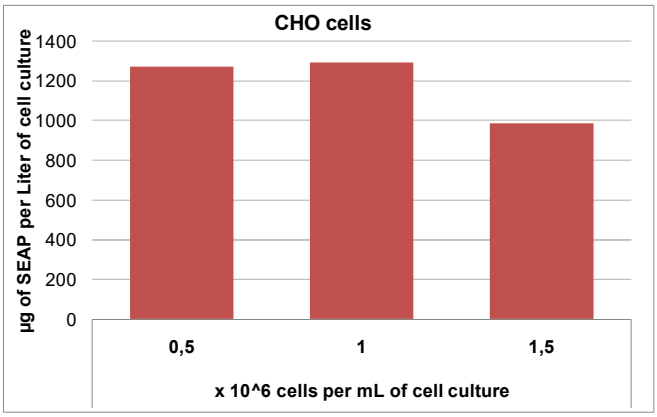


Fig.6: **Influence of DNA concentration on SEAP Expression with HYPE-5 Kit**
Transfections were performed using a range of DNA from 1 to 4 µg per milliliter of cell culture. Ratio for HYPE-5 reagent was fixed at 2µL per µg of DNA. Density of cell culture was fixed at 1 x 10^6 cells/mL.



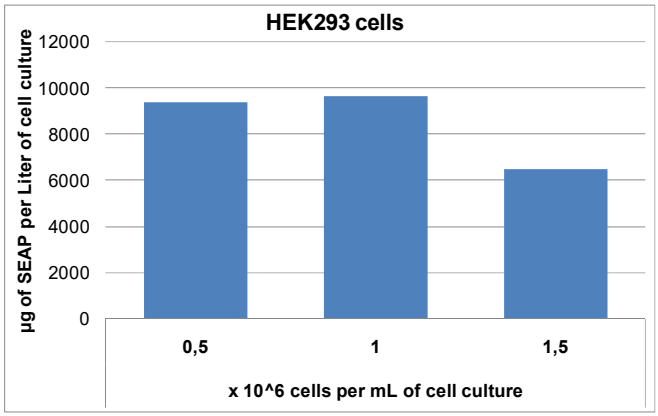


Fig.7: **Cell density influence on SEAP Expression with HYPE-5 Kit**
Transfections were performed using 1.5 µg per mL of cell culture. Ratio for HYPE-5 reagent was fixed at 2µL per µg of DNA. Density of cell culture was used in a range from 0.5 to 1.5 x 10^6 cells/mL the day of transfection.

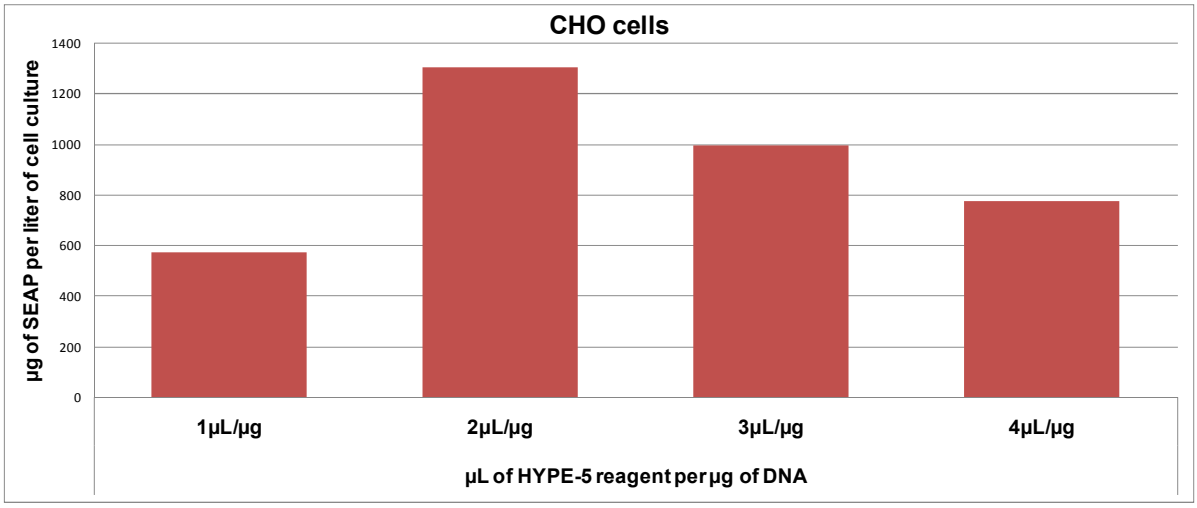


Fig.8: **Influence of HYPE-5 / DNA ratio on SEAP Expression.**
Transfections were performed using 1.5 µg per mL of cell culture. Ratio of HYPE-5 reagent was used in a range from 1 to 4µL per µg of DNA. Density of cell culture was fixed at 1 x 10^6 cells/mL the day of transfection.