The fast turnaround and cost-effectiveness of transient protein production provides an attractive option when limited quantities of research-grade protein are needed. For transient manufacturing, the gene of interest is introduced into host cells using an expression plasmid that contains all required genetic information for nuclear extra-chromosomal transcription and translation of the protein of interest. As a result, expression of the protein is lost after a limited number of cell divisions. Therefore, the harvest is usually done between 3-7 days post transfection. Key success determining parameters for effective deployment of this production method are high efficiency cell transfection and high cell viability before and after transfection.

Transient versus stable expression

The argument for choosing either transient or stable protein production hinges primarily around the question whether there is a need for a steady supply of the protein of interest with a narrow bandwidth on quality (purity, aggregation, etc). In general, stable protein production results in an indefinite, scalable supply of the protein and allows for the development of robust production and purification processes, in turn delivering highly comparable product quality after each production run. Therefore, in case of large scale, GMP quality production of therapeutic proteins, stable expression is preferred - read our STEP® technology fact sheet for more information on stable protein expression. In contrast, transient production is inherently less controlled due to quality differences in for instance DNA prep used (purity, supercoiling), cell division state at transfection and transfection procedure control. Therefore, transient expression strategies are usually selected during the drug discovery phase, when only small research quantities of the therapeutic protein are needed (10-1000 mg). Such quantities suffice to obtain quick proof of concept, ranging anywhere from questions on basic molecule design up to pharmacokinetics studies in pilot in vivo models or scouting experiments to assess biological activity and stability.

For transient transfection, both HEK293 cells and CHO cells are often used. Although usually HEK293 is the cell line of choice for mammalian transient transfection, due to their intrinsic ability to take up DNA, high levels of protein expression and robust cell culture processes, we often advise to utilize CHO cells instead. Approximately 70% of all licensed proteins are produced in stably transfected CHO cells. Therefore, it is deemed crucial to generate all pre-clinical data on the same cell platform. At the time of transfection, the CHO cells must have high viability (>95%) and should be in mid log phase. We found the optimal viable cell amount to be $2 \times 10^6$ per nucleofection ($2 \times 10^6$ in 105 µL during electroporation diluted down to $0.4 \times 10^6$ cells per mL after completion the transfection) at the time of transfection.

Transient production technology

At Batavia Biosciences, we use optimized processes for transient protein production. The transfection step is highly efficient by using techniques such as high cell density flow electroporation or complexing with PEI to rapidly and cost-effectively generate high quality proteins in sufficient quantities for research purposes. Success determining experimental parameters include higher than 90% transfection efficiency and higher than 90% cell viability after transfection.
Projects at Batavia are generally performed at small scale (20-50 mL) in high throughput mode (up to n=1000) for variant screening, or up to 50 L scale using in-house animal component free batch or fed-batch processes.

**Small scale, high throughput antibody production**

Batavia has developed a standard protocol for high throughput antibody manufacturing using transient protein technology. Hereo, we utilize a fully human antibody expression plasmid with a multiple cloning site in the CDR3 region that determines antibody specificity (for a schematic representation of the plasmid see Figure 1 below). Using this construct, target specific sequences can be rapidly inserted using multiple cloning sites resulting in generation of hundreds of different antibody expression plasmids all containing the same antibody scaffold. Using our standardized transfection method with PEI, a cell culture volume of 50 mL and 6×10⁶ viable cells per mL at transfection, we achieve between 1-10 mg purified antibody with a success rate of over 95% concerning yield (>1 mg), purity (>95%), aggregation percentage (<2%) and endotoxin level (<0.1 EU/mL). This success ratio drops to 50-80% when cloning complete cDNAs encoding for different light and heavy chains in our antibody scaffold expression plasmid. The turn-around time from the moment of receiving the DNA expression plasmids to delivering the antibody preps with a certificate of analyses is less than 1 month.

![Diagram of plasmid BAT-1 generating a complete IgG via 2 separate expression cassettes, one expressing the heavy chain (HC), and one expressing the light chain (LC).](image)

**Larger scale antibody production**

We have developed a standard protocol for larger scale antibody manufacturing using transient protein technology. Hereo, we use the MaxCyte system for high cell density transfection of up to 2×10¹⁰ viable cells per transfection. For the production of antibodies, we use disposable bioreactors up to 50 L and a generic batch production process void of any components derived from animals. Seven days after transfection, an average harvest yields up to 205 mg/L (SD 84 mg/L). With our optimized purification process using affinity chromatography and size exclusion chromatography a purity of >93.5% monomer is recovered. The turn-around time from the moment of receiving the DNA expression plasmids to delivering the antibody preps with a certificate of analyses is 1 month.

**Our transient production technology offers**

- Rapid protein (including antibody) production
- High production yields
- Cost-effective manufacturing of your product
- Optimized production and purification processes up to 50 L scale

for more information: [www.bataviabiosciences.com](http://www.bataviabiosciences.com)