Hera BioLabs | White Paper

Hera BioLab's CHO-K1 GS Double Knockout and Advanced Transposase Technology Deliver Higher Expressing Stable Pools for Therapeutic and Diagnostic Applications

Industry Leading Titers and Productivity Indicators Demonstrated with Trastuzumab

- >4 g/L titers in pools with standard media and low-cost workflows
- High productivity with >80 Qp
- Use of proprietary double GS KO CHO cell
- Powered by transposase technology
- < 1 Month turnaround

Traditional CHO cell workflows for downstream biomanufacturing are not compatible with early-stage programs nor budgets at innovator companies. Although transient protein expression systems are used during early-stage drug development, they generate small, variable quantities of proteins, complicating preclinical decision making. The optimization of engineered CHO hosts and efficient transposase technology have simplified the workflow and cost structure of producing lead biotherapeutics and antibody diagnostic products.

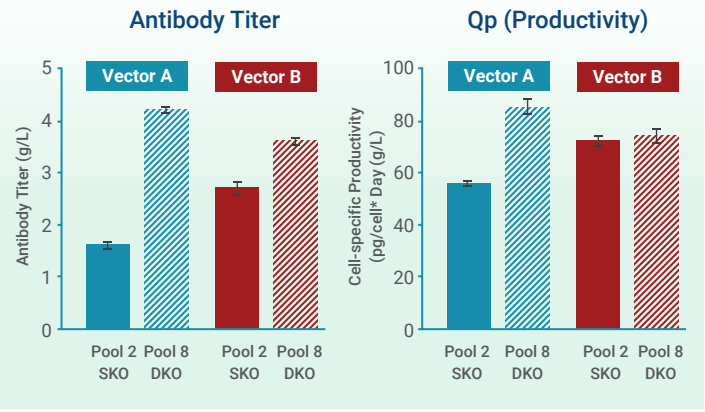
CHO pools expressing stable proteins are increasingly used in drug discovery for selecting lead biologics. In fact, CHO pools have been used to quickly produce antibodies with well-defined quality release criteria to support IND-enabling and early-stage clinical trials.

Hera Biolabs leverages best-in-class technologies, including our clonal double glutamine synthetase (GS) knockout (KO) CHO cell line, developed using Cas-CLOVER™, and our transposase-mediated integration system. Using these technologies, Hera can rapidly deliver high-producing CHO cell pools and cell lines at breakthrough costs. Hera has extensive experience in cell-line engineering, with continual investment in quality systems for cell culture, equipment monitoring, and single-cell cloning.

Hera has turnkey workflows to generate high-producing CHO pools through our unique double GS KO CHO cell line and transposase powered designer expression constructs. To demonstrate our workflow, trastuzumab was expressed in a comparative study using Hera's single and double GS KO CHO cell lines and proprietary vectors. To dial in GS selection with both the single (SKO) and double GS KO (DKO) cell lines, vectors included either a strong GS complementation cassette with a strong light chain promoter (Vector A) or a weak GS complementation cassette with a weak light chain promoter (Vector B). After nucleofection with Hera's transposase to catalyze transposition into the CHO genome and following selection, fed-batch cultures in glutamine deficient media were initiated in 24 deep well plates and titers, Qp, and quality metrics measured.

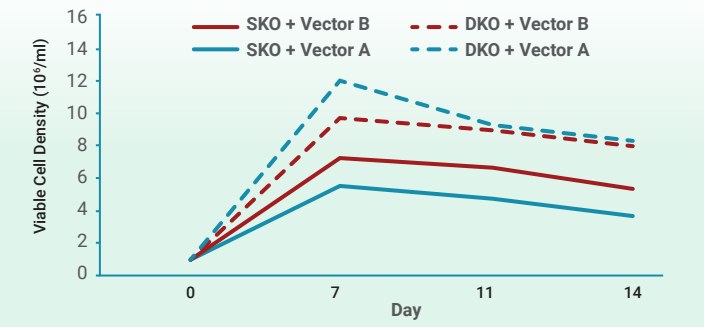
Industry Leading Titers and Productivity Indicators were Demonstrated with Trastuzumab

- All 4 pools achieved commercially advantageous titers (1.5-4.3 g/L) with Qps between 55-83 pg/cell*day using 24 deep well plates in a fed-batch system with off-the-shelf media.



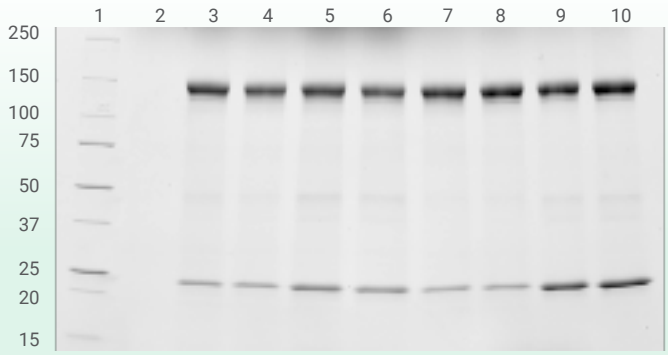
DKO was Superior to the SKO for Trastuzumab Production in Pools

- Qps for Vector B pools were similar in the two hosts, while Vector A Qps were higher in the double KO pool.
- Vector A: DKO pool achieved 2.3-fold higher viable cell densities versus the SKO pool.
- Vector B: DKO pool achieved 1.5-fold higher viable cell densities versus the SKO pools.



Rapid Quality Check Demonstrated Assembled Antibodies

- Both vectors and all 4 pools showed excellent antibody assembly by SDS PAGE (150kDa marker).
- Increasing promoter strength of the light chain led to excess light chain, as (25 kDa marker) observed in the conditioned media (lanes 5, 6, 9, and 10).



Conditioned medium containing 5 µg of protein was used for non-reducing SDS-PAGE.

Lane	Sample
1	Marker
2	Untransfected CM
3	SKO + Vector B
4	SKO + Vector B
5	SKO + Vector A

Lane	Sample
6	SKO + Vector A
7	DKO + Vector B
8	DKO + Vector B
9	DKO + Vector A
10	DKO + Vector A

Hera is now generating stable expression of antibody pools (>4 g/L). Our stable cell line development services leverage our elite CHO host systems and transposase technology for efficient transfection of genetically stable elements with unmatched selection capability. Our optimized workflow pairs transposase technology and vectorology with our easy to culture DKO GS CHO cells, leading to rapid, inexpensive, and scalable production of antibodies to support preclinical and IND-enabling studies. Conditioned media generated from these pools is suitable to begin downstream process and CMC development in conjunction with CDMO partners. CHO pools can undergo single-cell cloning at any time to identify select clones with growth and titers that exceed the pool averages.

Hera BioLabs

We Are Hera BioLabs

Hera Biolabs is leveraging cutting-edge gene editing technology, featured in 800+ peer-reviewed papers, to bring novel models and cell-line engineering to drug development researchers. See how our experienced team, US-based facilities, and portfolio of sophisticated tools and services accelerate drug discovery and development at www.herabiolabs.com.

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