

# *Expression of Recombinant Proteins in Mammalian and Avian Cell Lines*

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## **ABSTRACT**

Mammalian and avian cell lines are increasingly used in recombinant protein production because of their capacity to produce physiologically active proteins with precise post-translational modifications. Mammalian systems such as CHO and HEK293 are commonly utilised to produce therapeutic proteins and monoclonal antibodies; avian cell lines are gaining popularity in vaccine manufacturing due to their quick growth and effective viral replication. Advances in cell line engineering and hybrid expression platforms are increasing productivity, scalability, and biomanufacturing efficiency.

## **INTRODUCTION**

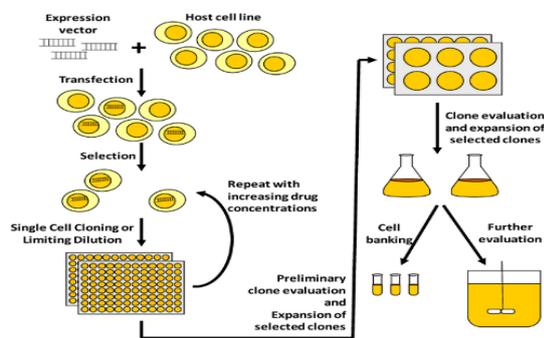
**R**ecombinant protein manufacturing is one of modern biotechnology's most potent tools, allowing for large-scale synthesis of therapeutic proteins, vaccines, monoclonal antibodies, hormones, and diagnostic reagents. While bacteria and yeast were once the predominant expression hosts, mammalian and avian cell lines have become the systems of choice for creating

physiologically active and therapeutically safe biologics (Zhu *et al.*, 2017). Unlike microbial hosts, mammalian and avian cells are closely related to human and animal tissues and have the cellular machinery needed for proper protein folding, assembly, and post-translational modifications (PTMs), such as glycosylation, phosphorylation, and disulphide bond formation. These changes are necessary

for protein stability, biological activity, and decreased immunogenicity. As a result, mammalian and avian cell lines are currently used extensively to produce vaccines, monoclonal antibodies, cytokines, and antiviral proteins for human and veterinary medicine, and they are well-accepted by global regulatory agencies.

### How Recombinant Protein Expression Works?

Through the process of recombinant protein expression, scientists can "instruct" cells to manufacture a certain protein, even if the cell is not ordinarily capable of doing so. Because they may produce proteins that closely mimic real human or animal proteins, mammalian and avian cell lines are especially important.



**Fig 1:** Standard process for creating a recombinant mammalian cell line: The expression vector containing the gene of interest (GOI) and a selection marker is transfected into host cells, followed by drug selection and cloning to produce GOI-expressing cells. Gene amplification with increasing doses of selection agents (e.g., MTX or MSX) increases productivity. High-producing clones are then chosen, expanded, and tested for protein yield, stability, and quality before being banked (Lai *et al.*, 2013).

### Mammalian Cell Lines: The Most Popular Choice

Mammalian cell lines are the backbone of the biopharmaceutical industry due to their ability

to produce human-like proteins with accurate PTMs(Lai *et al.*, 2013).

#### 1. CHO Cells: The Industry Workhorse-

CHO (Chinese Hamster Ovary) cells are the most frequently used mammalian expression system, accounting for roughly 70-80% of all FDA-approved biologic drugs such as cytokines, clotting factors, and monoclonal antibodies. Their broad use stems from their capacity to grow efficiently in suspension cultures ideal for large-scale bioreactors, adapt to serum-free and chemically defined medium, and endure changes in pH, nutrients, osmolality, and oxygen levels. CHO cells are also highly genetically stable, which is essential for reliable monoclonal antibody production, and have a lengthy history of regulatory acceptability. Furthermore, new fed-batch techniques allow CHO cells to produce very high protein yields of 5-10 g/L, making them economically and industrially appealing for biopharmaceutical manufacture.

#### 2. HEK293 Cells: Ideal for Complex and Viral Proteins-

HEK293 cells, generated from human embryonic kidney tissue, have very human-like PTMs and are ideal for generating complex proteins, membrane receptors, and viral antigens. Their excellent transfection effectiveness and compatibility with viral vector generation make them indispensable for gene therapy, vaccine research, and recombinant viral particle manufacture.

#### 3. NS0 and Other Myeloma Cells for Antibodies-

NS0 cells, generated from mouse myeloma, are specialised for antibody secretion and are commonly employed in monoclonal antibody synthesis. Their capacity to maintain appropriate heavy and light chain assembly, folding, and secretion has led to their

continued usage in therapeutic antibody production.

### Avian Cell Lines: An Emerging Platform

Avian cell lines include EB66, DF-1, and AGE1.CR is becoming increasingly important, notably in viral vaccine manufacturing. These cells grow quickly, require simple culture conditions, and respond well to serum-free media. Importantly, avian cells offer a controlled alternative to traditional egg-based vaccine production, avoiding concerns such as seasonal egg availability, egg-adapted virus changes, and contamination risks. Avian cell lines have advantageous glycosylation profiles, low immunogenicity, and high viral replication efficiency, making them increasingly appealing for recombinant vaccine and diagnostic protein synthesis (Farzaneh *et al.*, 2017). Examples of vaccines produced in avian cells are shown in Table 1:

**Table 1: Examples of vaccines produced in avian cells**

S.No.	Vaccine Type	Cell Line Used
1.	Influenza (H1N1, H3N2, H5N1)	EB66, AGE1.CR
2.	Newcastle Disease Virus (NDV)	DF-1
3.	Avian Influenza Vaccines	EB66
4.	Japanese Encephalitis (partially)	Avian-derived substrates

**Table 2: Comparison between Mammalian vs Avian Expression Systems**

Sl. No	Parameter	Mammalian Expression Systems	Avian Expression Systems
1.	Cell Origin	Mammalian species (hamster, human, mouse)	Avian species (duck, chicken)
2.	Growth Rate	Moderate; slower doubling time	Faster doubling time; shorter production cycles
3.	Culture Conditions	Complex; often require	Simpler; adapt well to serum-

		optimized media	free, defined media
4.	Post-Translational Modifications (PTMs)	Highly accurate, human-like; ideal for therapeutics	Simpler, cleaner glycans; lower risk of allergenicity
5.	Glycosylation Pattern	Human-like but sometimes heterogeneous	More uniform, consistent glycosylation patterns
6.	Protein Production Yield	High but depends on line (e.g., CHO high yield)	High; especially efficient for viral proteins and vaccines
7.	Support for Viral Replication	Excellent for human viruses; used for viral vectors	Natural support for avian & human viruses; ideal for flu vaccines
8.	Transfection Efficiency	High (especially HEK293)	Moderate to high depending on cell line
9.	Scalability	Widely optimized for large bioreactors (up to 20,000 L)	Very scalable; good performance in single-use bioreactors
10.	Regulatory Acceptance	Very well-established; decades of FDA/EMA/WHO approval	Increasingly accepted, especially for vaccines
11.	Contamination Risk	Low, but requires strict control	Lower compared to egg-based vaccine systems
12.	Production cost	Higher due to the media and slower growth	Lower due to rapid growth and simpler media
13.	Typical Applications	Monoclonal antibodies, hormones, enzymes, cytokines, gene therapy vectors	Influenza vaccines, veterinary vaccines, and certain recombinant proteins
14.	Examples	CHO, HEK293, NS0	EB66, DF-1, AGE1.CR

### Applications of Recombinant Proteins

These expression systems are required to produce therapeutic monoclonal antibodies (e.g., Rituximab and Trastuzumab), vaccinations for human and veterinary illnesses, cytokines, enzymes, and growth factors. They also contribute to breakthroughs in personalised medicine, disease models, gene

therapy, and protein engineering. Mammalian and avian cell lines continue to affect the future of biotechnology, healthcare, and vaccine research by allowing the creation of biologics that closely resemble natural proteins.

### Future Prospects

Future developments will increasingly rely on designed and hybrid expression platforms that combine high productivity with human-like post-translational modifications. These next-generation cell lines, aided by breakthroughs in systems biology, artificial intelligence, and high-throughput screening, are projected to accelerate, secure, and customise biomanufacturing, ultimately enhancing worldwide access to biologics, vaccines, and precision medicine.

### CONCLUSION

Mammalian and avian cell lines are critical platforms for recombinant protein and vaccine synthesis because they can produce physiologically active proteins with precise post-translational modifications. Mammalian systems dominate therapeutic protein manufacture, whereas avian cell lines provide cost-effective, scalable vaccine production

options. Continued breakthroughs in cell line engineering will increase productivity, safety, and global access to biologics.

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