**BalanCD® HEK293 SYSTEM**

**Maximize productivity in suspension cultures**

- Versatile formulation supports transfection and production including:
  - Rapid, scalable production of viral vectors
  - Use the same system for a wide range of HEK293 cell lines
  - Increased productivity in transient protein expression

- Fulfills regulatory requirements:
  - Chemically-defined, animal-component free
  - Drug Master File (DMF) filed with US FDA

BalanCD® HEK293 is a scalable system designed to support growth and transfection of HEK293 cell lines in suspension cultures. Comprised of BalanCD HEK293 medium, BalanCD HEK293 Feed and Anti-Clumping Supplement, this highly versatile system supports a range of applications including production of viral vectors for gene therapy, transient protein expression and recombinant protein production. BalanCD HEK293 is part of the scalable BalanCD media and supplements platform designed to provide the optimal balance between growth and production to maximize productivity from mammalian cell cultures.

**Use the same growth medium at every step and every scale**

**Maintain cell stocks**

BalanCD HEK293

(Anti-Clumping Supplement optional)

**Seeding** → **Transfection** → **Production**

**Viral transfection and expression**

BalanCD HEK293

(Anti-Clumping Supplement optional)

**Transient expression and protein production**

BalanCD HEK293

BalanCD HEK293 Feed

(Anti-Clumping Supplement optional)
**Rapid, scalable production of viral vectors**

- Achieve optimal production yields within 48 hours
- Use the same medium for transfection and production
- Add Anti-Clumping Supplement to prevent aggregation

**SIMPLE, SCALABLE PROTOCOL SUPPORTS CELL GROWTH AND INCREASES YIELD OF LENTIVIRAL VECTOR**

![Graph showing cell density and titer over time](image1)

**Figure 1.** HEK293T cells were cultured in BalanCD HEK293 medium in a 2L working volume bioreactor and passaged every 3–4 days. PEI-mediated transfection of 4 plasmids was performed 3 days after seeding at a DNA:PEI ratio of 1:1.5. Viable cell density was determined. Lentivirus titer was measured at 24, 48, and 72 hours post-transfection.

**IMPROVED PRODUCTIVITY OF ADENO-ASSOCIATED VIRUS (AAV) COMPARED TO A COMMERCIALLY-AVAILABLE MEDIUM**

![Graph showing cell diameter and AAV titer over time](image2)

**Figure 2.** HEK293VC cells were cultured in a 2L working volume bioreactor. PEI-mediated transfection of 3 plasmids was performed 3 days after seeding at a DNA:PEI ratio of 1:1.5. Cell diameter were determined. AAV titer was measured at 48, 72, and 96 hours post-transfection.

Bioreactor data courtesy of Généthon, Evry Cedex, France.
Increase productivity in transient protein expression

- Achieve gram-scale yields of proteins using a simple scalable protocol
- Grow, transfect and produce in BalanCD HEK293 medium
- Maximize yield by adding BalanCD HEK293 Feed and Anti-Clumping Supplement post-transfection

TWO-FOLD INCREASE IN TITER COMPARED TO OTHER COMMERCIALLY-AVAILABLE MEDIA

Figure 3. HEK293-6E cells were cultured in duplicate 125mL Erlenmeyer flasks with a 30 mL working volume. Cells were seeded at 3 \times 10^5 \text{ cells/mL} in BalanCD HEK 293 medium. PEI-mediated transfection was performed on day 3 to produce a biosimilar antibody. BalanCD HEK293 medium was supplemented with a 5% v/v addition of BalanCD HEK293 Feed on days 1-4 post-transfection. Protein titer was measured on days 7, 9, and 11 post-transfection. Cultures were terminated when viability dropped below 70%, (data not shown). Data shown represents the average of two duplicate cultures.
Use the same system for a wide range of HEK293 cell lines

- Achieve higher cell densities while maintaining viability
- Increase culture longevity and maximize growth by adding BalanCD HEK 293 Feed
- Add Anti-Clumping Supplement to prevent aggregation

**COMPARISON WITH OTHER COMMERCIALY-AVAILABLE MEDIA**

Figure 3. Three different commercially available HEK293 cell lines were cultured in 125mL shaker flasks, at a working volume of 20-25%. Cells were seeded at $3 \times 10^5$ cells/mL, in duplicate, BalanCD HEK293 medium, supplemented with Anti-Clumping Supplement, and fed on days 3, 4, 5, and 6, at 5% v/v. Data shown represents the average of two duplicate cultures.

FreeStyle and Expi293-F are trademarks of ThermoFisher Scientific.; HEK292.2sus from ATCC
With you at every step

From development through to large-scale production, success is highly dependent on providing the optimal culture conditions. The BalanCD platform of cell-specific growth media and feeds has been developed to avoid many of the challenges faced during cell line development through process optimization and into commercial production. These serum-free, chemically-defined media and their supplements provide the optimal balance between growth and production to maximize productivity at any scale.

Media optimization and customization

Beginning with the most suitable BalanCD medium reduces development and optimization times required to achieve the required yield and quality of end-product. For those requiring customization to meet a specific need, experienced Irvine Scientific professionals can provide efficient, cost-effective and time-saving assistance.

Rapid prototyping

Normally provided within 10 working days, a rapid prototyping service offers flexible, small-scale, non-GMP media production of liquid and powder formulations. By using the same raw materials sourced and quality-controlled as for our large-scale GMP manufacturing, this approach greatly facilitates the step from research to process development activities.

A smooth transition into commercial production

Irvine Scientific products and services are developed according to the highest medical standards. Every BalanCD product is subject to a stringent Quality System unrivalled in the industry. To fulfill quality and reliability requirements, proprietary and customized media are manufactured under fully cGMP compliant conditions in dedicated animal-component free, state-of-the-art facilities replicated in California and Japan. Comprehensive documentation, including information from Supply Chain Management through to Drug Master Files, is available to help minimize the regulatory burden.
## Ordering Information

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*Custom sizes and packaging available on request.