BalanCD HEK293 Viral Feed

Catalog #	Product	Format	Available Package Sizes*
91176	BalanCD HEK293 Viral Feed	Liquid	500 mL
981921**	BalanCD HEK293 Viral Feed	Powder	10 L

* Additional package sizes are available upon request
** Available upon request

Intended Use

For research or further manufacturing use only.

Product Description

BalanCD HEK293 Viral Feed is a chemically defined, animal component-free medium optimized to enhance viral vector production in HEK293 cells. It contains no hydrolysates, L-Glutamine, antibiotics, antimycotics, or any other undefined components, and is a ready-to-use feed supplement for HEK293 suspension culture post-transfection. BalanCD HEK293 Viral Feed contains 40 g/L glucose. It is optimized to use with BalanCD HEK293 medium and compatible with other basal growth media for HEK293 suspension cultures. BalanCD HEK293 Viral Feed can be supplemented in cultures along with Anti-Clumping Supplement to minimize cell aggregation.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis, which is available at www.irvinesci.com or upon request.

Storage Instructions and Stability

LIQUID MEDIUM

Handle using aseptic techniques to avoid contamination. Store at 2-8°C and protect from light. This product is stable for 12 months, when unopened and stored properly. Do not use after the assigned expiration date. Not validated for use beyond the unopened expiry shelf life. Do not use any bottle of medium that shows evidence of particulate matter or cloudiness.

POWDER MEDIUM

Store dry at 2-8°C protected from moisture in the atmosphere. This product is very hygroscopic and should be kept in a dry environment away from moisture. Bring the powder to room temperature before opening and re-seal tightly after use. The powder should be free flowing; do not use if it is caked. This product is stable for 24 months, when unopened and stored properly. Do not use after the assigned expiration date.

Directions for Use

HYDRATION OF BALANCD HEK293 VIRAL FEED FROM POWDER MEDIUM

1. Add powder medium (130.03 g/L, PN #981921) to WFI (Q.S. to 1000 mL/L, Catalog #9309 or equivalent) into an appropriately sized container.
2. Mix the solution approximately 30-60 minutes or until the powder is well dissolved (the solution may still appear cloudy at this point). 5N HCL may be added dropwise to bring the pH down to slight acidic conditions for complete dissolution.
3. Add 2.20 g/L Sodium Bicarbonate to the solution and mix at moderate speed until completely dissolved.
4. Measure pH and adjust it to the expected range 6.9-7.4 using 5N NaOH solution. The expected osmolality range is 1100-1500 mOsm/kg.
5. Sterile filter through a 0.2µm filter membrane.
6. The solution can be stored in the dark at 2-8°C for up to 1 year.
7. Supplement 20 mL/L of 200 mM L-Glutamine (Catalog #9317) to BalanCD HEK293 Viral Feed to reach 4 mM final concentration prior to use, if necessary.
8. BalanCD HEK293 Viral Feed contains 0.1% Poloxamer; however, an additional 0.05% to 0.1% can be supplemented, if necessary.

- **Note:** For cell recovery and adaptation, sub-culturing, and cryopreservation please refer to the Product Insert for BalanCD HEK293 system (Catalog #91165 and 94137), available at www.irvinesci.com.

Feeding and Supplementations

Cells cultured in BalanCD HEK293 medium (Catalog #91165 or 94137 with 4 mM of L-Glutamine, Catalog #9317) can be supplemented with BalanCD HEK293 Viral Feed to boost viral vector production.

Anti-Clumping Supplement (Catalog # 91150) may be added to cultures if cells start to aggregate. For cultures where this supplement is added, Anti-Clumping Supplement must be eliminated from the culture media prior to transfection, as this supplement will completely inhibit transfection. To remove Anti-Clumping Supplement from culture, spin down cells, then wash cells with either 1X PBS (Catalog # 9240), or BalanCD HEK293 medium (catalog # 91165). Re-suspend cells in BalanCD HEK293 medium *without* Anti-Clumping Supplement before proceeding with transfection.

- **Note:** BalanCD HEK293 Viral Feed is intended to use post transfection, as it will completely inhibit transfection.

Recommended Protocol

1. Use cells in the logarithmic phase of growth (1 to 5 X 10^6 cells/mL) that have been adapted into BalanCD HEK293 medium supplemented with 4 mM of L-Glutamine (Catalog #9317).
2. Seed cells at 1x10^6 viable cells/mL a day prior transfection (day -1).
3. Transfect cells at day 0 with a mixture of plasmid vectors and a transfection reagent, following the recommended protocol offered by the transfection reagent manufacturer (optimal transfection parameters vary based on a number of factors including but not limited to cell type and DNA purity. Therefore, user-specific optimization of parameters is highly encouraged). **Cell viability at transfection should be >90% with viable cell density of 1.5 – 2.5 X 10^6 cells/mL.** Count cells after transfection to collect the baseline data.
4. Add **12% (v/v) BalanCD HEK293 Viral Feed (Catalog #91176)** to total culture volume about 24 h post-transfection (day +1).
5. Optional: Collect sample of cells for transfection efficiency measurement (Using FACS or any image-based method), 48 h post-transfection (day +2).
6. Collect samples for cell count and viral titer measurement every day starting at day +1 to find the optimum harvest time (Day +2 to day +4).
7. Perform metabolite measurements (pH, osmolality, L-glutamine, Glucose, Lactate, Ammonium) at transfection (day 0) and monitor them at the harvest time.

8. Collect samples (500 µL/sample) at transfection and at the harvest time for future spent media analysis. Cells need to be clarified, media needs to be filtered through either 0.1 or 0.2 µm filter, and stored at -10 °C.

BalanCD HEK293 Viral Feed Optimization Guideline

BalanCD HEK293 Viral Feed can be evaluated with the suggested feed method mentioned above. However, optimization of feed schedule and volume is highly encouraged to achieve optimal culture performance and results. The starting time of feeding should be at least 6 hours, post-transfection to avoid any interference with transfection. The total feed can range 10-15% and can be applied at single bolus or in multiple feeding time points.

- **Note:** The end user should optimize feeding schedule and conditions, including titering concentration by monitoring cell health indices.

Example Applications Data

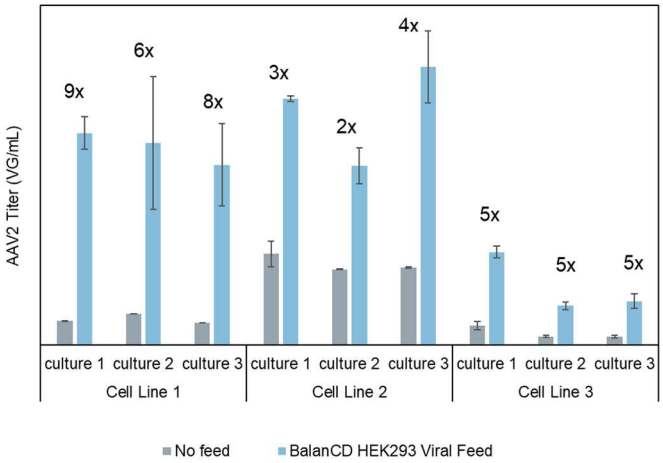
Adeno-Associated Virus (AAV) production

The following PEI-mediated transient transfection protocols can be used as a general guideline to begin optimization of transfection method. Optimization of transfection parameters is highly encouraged, since optimal transfection parameters may vary depending on the application.
BalanCD HEK293 medium is also compatible with other transfection methods using cationic, liposomes or electroporation.

PEI-mediated transfection

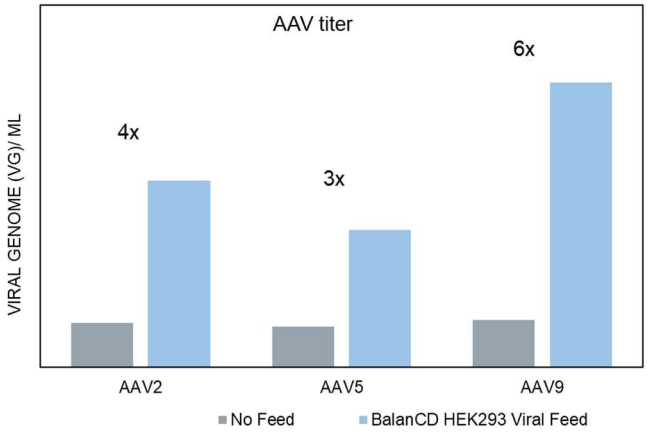
CULTURE CONDITIONS USED IN THIS EXAMPLE

- Medium: BalanCD HEK293 (Catalog #91165) supplemented with 4mM L-Glutamine (Catalog #9317)
- Cell stock: HEK293 cells directly thawed into BalanCD HEK293 medium supplemented as stated above, and passaged every 3 to 4 days for three passages before transfection
- Culture vessel: Corning 125-mL flat-bottom polycarbonate shake flask with vent cap
- Working volume: 20-25% of flask volume (i.e. 25-30 mL in 125-mL flask)
- Seeding density for passage: 3 x 10^5 cells/mL
- Seeding density for transfection: ~1 x 10^5 cells/mL, a day before transfection
- Cell density at transfection: ~2 x 10^6 cells/mL
- Incubator setting: 37°C, 5% CO_2, 120 rpm shaking speed, 80% humidity
- Transfection on Day 0 as described above:
    - >90% Viability
    - Transfection agent: Polyethylenimine, PEIpro (PolyPlus)
    - 3 plasmid for AAV (molar ratio, 1:1:1)
    - DNA:PEI ratio at 1:2
    - Total amount of 1.5 µg DNA per mL culture
- **Feed: 24 h post-transfection,12% (v/v) of culture using BalanCD HEK293 Viral Feed**

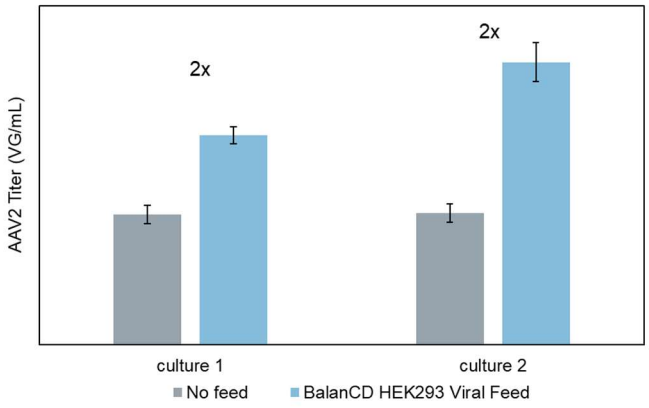


BalanCD HEK293 Viral Feed boosts AAV2 production in various HEK293 lines. HEK293 cells (three cell lines) were cultured in triplicate (culture 1-3) using BalanCD HEK293 medium and supplemented with 12% (v/v) BalanCD HEK293 Viral Feed at 24 h post-transfection or no feed control. The cells were harvested 72hr post-transfection. The AAV2 titer in each cell culture was quantified by qPCR using AAV real-time PCR titration kit (TakaraBio) for cell line 1 and 2, and digital PCR (QIAcuity) for cell line 3. In all cases, ITR primers were used. The error bars show standard deviation of qPCR triplicates for each cell lysate sample. Fold titer improvement over no feed control is shown above each condition set.

BalanCD HEK293 Viral Feed increases AAV titer in multiple different AAV serotypes. HEK293 cells (Cell line 4) were cultured in BalanCD HEK293 medium, transfected with viral vectors expressing different AAV serotypes, and supplemented with BalanCD HEK293 Viral Feed or no feed control. 1 µg DNA per mL culture in 300 mL culture volume was used. AAV titer was measured by digital droplet PCR (Biorad). Fold titer improvement over no feed was indicated for each serotype.



Lipid-Mediated Transfection



BalanCD HEK293 Viral Feed is compatible with lipid-based transfection. HEK293 cells (Cell line 2, representing cultures in duplicate) were grown in BalanCD HEK293 medium and transfected using AAVmax (Thermo Fisher Scientific) transfection kit material and method, including cationic lipid-based transfection reagent, transfection booster and AAV production enhancer according to kit protocol. BalanCD HEK293 Viral Feed or No feed control was added as recommended. Viral genome titer measured by dPCR (QIAcuity) using ITR primers indicated that BalanCD HEK293 Viral Feed could increase AAV2 production 2X fold over No feed control.

Related Products

Catalog #	Product	Available Package Sizes
91165	BalanCD HEK293, Liquid	1 L
94137	BalanCD HEK293, Powder	10 L
91150	Anti-Clumping Supplement	50 mL
9317	L-Glutamine Solution (200 mM)	100 mL, 500 mL
9309	Water for Injection (WFI)	1L, 20L, 200 L
9240	1X PBS, Dulbecco's Phosphate Buffered Saline	100 mL, 500 mL, 1 L

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: fisitmrequest@fujifilm.com
- Direct line: US +1 800 577 6097
  EU +31 13 5791911

WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- Safety Data Sheets (SDS)
- COAs (when available)
- FAQs (when available)
- Product literature
- Complete list of offices and contact information by country

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