BalanCD HEK293 System

Catalog #	Product	Format	Available Package Sizes**
91165	BalanCD HEK293	Liquid	1 L
94137	BalanCD HEK293	Powder	10 L
91166	BalanCD HEK293 Feed	Liquid	500 mL
91150	Anti-Clumping Supplement	Liquid	50 mL

**Additional package sizes are available upon request

Intended Use

For research or further manufacturing use only.

Product description.

The BalanCD HEK293 System is a chemically defined, animal component-free platform of media and supplement optimized for production of viral vectors and transient protein expression. The product system comprises of BalanCD HEK293 medium, BalanCD HEK293 Feed, and Anti-Clumping Supplement. BalanCD HEK293 System contains no hydrolysates, L-Glutamine, antibiotics, antimycotics, or any other undefined components, and is ready to use for suspension culture applications. BalanCD HEK293 medium contains 6 g/L glucose and BalanCD HEK293 Feed contains 40 g/L glucose. BalanCD HEK293 medium can be supplemented with BalanCD HEK293 Feed for high density cell culture, or with Anti-Clumping Supplement post-transfection 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 FROM POWDER MEDIUM

1. Add powder medium (21.32 g/L, Catalog #94137) to WFI (1000 mL/L, Catalog #9309 or equivalent) into an appropriately sized container.
2. Mix the solution approximately 10 minutes or until the powder is well dissolved (the solution may still appear cloudy at this point).
3. Add 2.20 g/L Sodium Bicarbonate to the solution and mix at moderate speed until completely dissolved.
4. Measure pH (expected range 6.7-7.4) and osmolality (expected range 280-320 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 medium to reach 4 mM final concentration prior to use.
8. BalanCD HEK293 contains 0.1% poloxamer; however, an additional 0.05% to 0.1% can be supplemented if necessary.

CELL RECOVERY AND ADAPTATION

1. Supplement BalanCD HEK293 medium (Catalog # 91165 or 94137) with 4mM L-Glutamine (Catalog #9317). Aseptically transfer appropriate volume (30 mL) of supplemented medium into a baffled 125 mL shake flask and equilibrate in a 37ºC, 5% CO_2 incubator.
2. Thaw frozen vial rapidly in a 37°C water bath.
3. Transfer the cells to a culture flask with pre-equilibrated BalanCD HEK293 medium (from step 1) to achieve an initial cell density of 3 x 10^5 cells/mL.
4. Incubate culture in a 37ºC, 5% CO_2 incubator for 3 to 4 days.
5. Sub-culture cells following the Sub-culturing Procedure outlined below.
   NOTE: Cells can be directly adapted into BalanCD HEK293. After a minimum of three passages in BalanCD HEK293, if cells have successfully adapted, viable cell density and percent viability should reach above 1.5 x 10^6 cells/mL, and 90%, respectively.
6. If severe cell aggregation is observed, continue passaging with the following recommendation:
   a. Supplement 2 mL/L Anti-Clumping Supplement (Catalog #91150).
7. If cells grow slowly (less than 1 x 10^6 cells/mL within 4 days) with viability below 90%, continue passaging with the following recommendations:
   a. Increase seeding density to 0.5-1 x 10^6 cells/mL.
   b. Spin down and re-suspend cells into fresh medium at each passage.
   c. Sequential adaption at ratios of 1:1, 1:2, 1:4, and 0:1 current medium to BalanCD HEK293.

SUB-CULTURING PROCEDURE

1. Supplement BalanCD HEK293 medium (Catalog #91165 or 94137) with 4 mM of L-Glutamine (Catalog #9317).
2. Sub-culture cell stocks every 2 to 3 days to keep cells in early logarithmic growth phase. Seed at a density of 3-5 x 10^5 cells/mL. Viable cell density and percent viability should reach above 1.5 x 10^6 cells/mL and 90%, respectively within 3 days.
   Note: It is strongly recommended to keep the cultures under 3 x 10^6 cells/mL in order to achieve high transfection efficiencies.
3. If severe cell aggregation is observed, continue passaging with the following recommendations:
   - Maintain viable cell density below 2 x 10^6 cells/mL.

- If problem still persists, add 2 mL/L of Anti-Clumping Supplement (Catalog #91150).

Note: Anti-Clumping Supplement is designed for use at a dilution between 1:1000 (1 mL/L) and 1:100 (10 mL/L) depending on degree of clumping.

CRYOPRESERVATION

1. Prepare required volume of freezing medium (90% cold BalanCD HEK293 + 10% DMSO). Keep at 4°C until ready to use.
2. Centrifuge appropriate number of healthy cells for 5 minutes at 200g and decant or aspirate the supernatant without disturbing the cell pellet.
3. Re-suspend cells in cold freezing medium at a density of 1 x 10^7 viable cells/mL (or other desired cell density based on user needs).
4. Aliquot 1 mL/vial (or desired volume) into sterile cryovials.
5. Gradually lower the temperature of the vials to -80°C at a rate of -1°C/minute in an appropriate freezing container.
6. Once cells reach -80°C, transfer to liquid nitrogen vapor phase for long term storage.

Feed and Supplement for BalanCD HEK293

BalanCD HEK293 medium can be supplemented with BalanCD HEK293 Feed and/or Anti-Clumping Supplement to support multiple applications utilizing HEK293 cells. Anti-Clumping Supplement 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). Resuspend cells in BalanCD HEK293 medium *without* Anti-Clumping Supplement before proceeding with transfection.

Purpose	BalanCD HEK293 Feed **(Catalog #91166)**	Anti-Clumping Supplement **(Catalog #91150)**
Cell stock sub-culturing	No*	Optional: add 1-2 mL/L, if cells begin to aggregate
Post-transfection enhancement of cell growth and protein yield	Yes See section: BalanCD HEK293 Feed Optimization Guideline	Optional: add 1-2 mL/L at least one day *post transfection*, if cells begin to aggregate
Viral vector production	No*	Optional: add 1-2 mL/L at least one day *post transfection*, if cells begin to aggregate
Enhancement of stably transfected cell growth and recombinant protein yield	Yes See section: BalanCD HEK293 Feed Optimization Guideline	Supplement 1 mL/L to BalanCD HEK293 medium prior to use

**BalanCD HEK293 Feed is recommended when extending the culture duration for more than a week and is not recommended for use for cell stock sub-culturing or viral vector production.*

BalanCD HEK293 Feed Optimization Guideline

BalanCD HEK293 Feed can be evaluated with the *suggested standard feed method* shown below. However, optimization of feed schedule and volume is highly encouraged to achieve optimal culture performance.

EXPRESSION BY TRANSIENT TRANSFECTION

1. Growth Medium: BalanCD HEK293 with 4 mM L-Gln
2. Determine optimal feed volume. Evaluate total feed volume at a range of 12-20% as below.
   %Feed volume = % of initial culture volume.

Day Post Transfection				Total Feed Volume
1	2	3	4	
3%	3%	3%	3%	12%
4%	**4%**	**4%**	**4%**	**16%***
5%	5%	5%	5%	20%

***Suggested standard feed method**

3. Determine feed schedule using feed volume determined from above in step 2. Feeding can be split into 4 events at an equal volume. Example with 20% total volume shown.

Day Post Transfection							
1	2	3	4	5	6	7	8
5%	5%	5%	5%				
	5%	5%	5%	5%			
		5%	5%	5%	5%		
			5%	5%	5%	5%	
5%		5%		5%		5%	
	5%		5%		5%		5%

EXPRESSION BY STABLE TRANSFECTION

1. Growth Medium: BalanCD HEK293 with 4 mM L-Gln and 1 mL/L Anti-Clumping supplement
2. Determine optimal feed volume: Evaluate total feed volume at a range of 10-25% as below.
   %Feed volume = % of initial culture volume.

Culture Day					Total Feed Volume
3	**4**	**5**	**6**	**7**	
2%	2%	2%	2%	2%	10%
3%	3%	3%	3%	3%	15%
4%	**4%**	**4%**	**4%**	**4%**	**20%***
5%	5%	5%	5%	5%	25%

***Suggested standard feed method**

3. Determine feed schedule using feed volume determined from above in step 2. Feeding can be split into 5 events at an equal volume. Example with 20% total volume shown.

Culture Day									
3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
4%	4%	4%	4%	4%					
	4%	4%	4%	4%	4%				
		4%	4%	4%	4%	4%			
			4%	4%	4%	4%	4%		
4%		4%		4%		4%		4%	
	4%		4%		4%		4%		4%

Example Applications Data

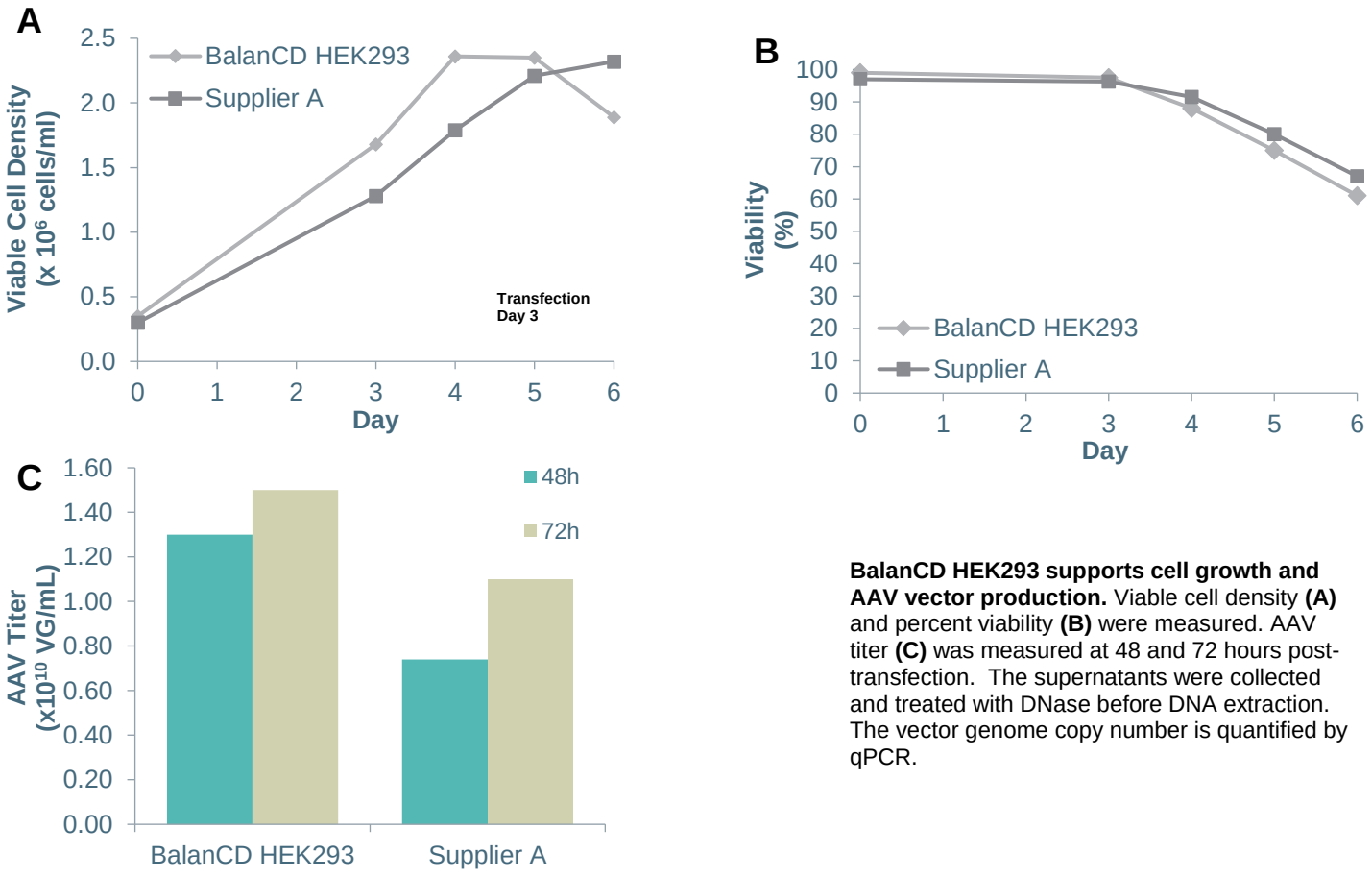
PEI-Mediated Transfection

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.

AAV Production

CULTURE CONDITIONS USED IN THIS EXAMPLE

- Medium: BalanCD HEK293 supplemented with 4mM L-Glutamine and 0.1% Poloxamers
- 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 going into the bioreactor production run
- Culture vessel: Benchtop Bioreactor with 2L working volume
- Seeding density: 3 x 10^5 cells/mL
- Incubator setting: 37°C, 5% CO_2, humidified
- Transfection on Day 3
    - >90% viability
    - Transfection agent: Polyethylenimine (PEI)
    - 3 plasmids for AAV vector
    - DNA:PEI ratio at 1:1.5
    - Total amount of 2µg DNA per 10^6 cells

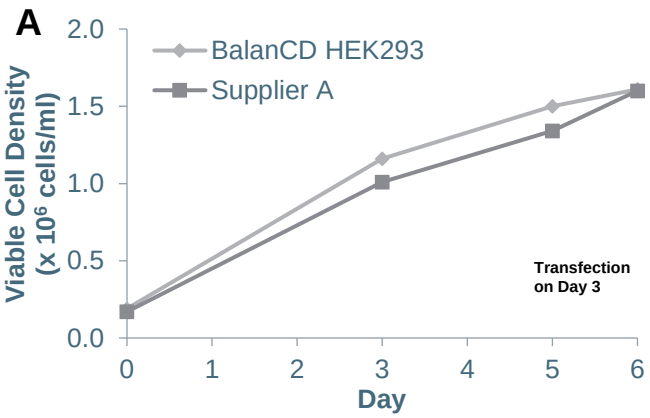


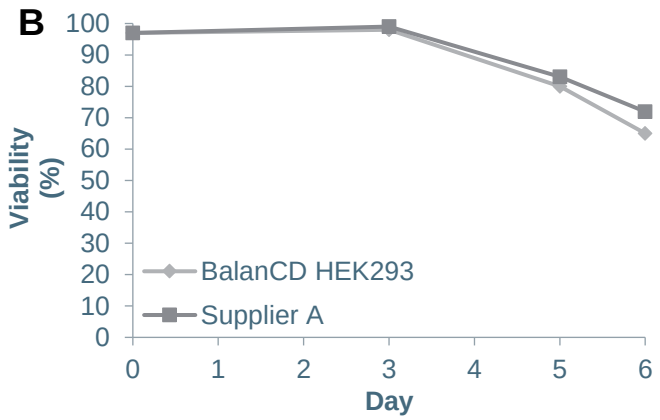
BalanCD HEK293 supports cell growth and AAV vector production. Viable cell density **(A)** and percent viability **(B)** were measured. AAV titer **(C)** was measured at 48 and 72 hours post-transfection. The supernatants were collected and treated with DNase before DNA extraction. The vector genome copy number is quantified by qPCR.

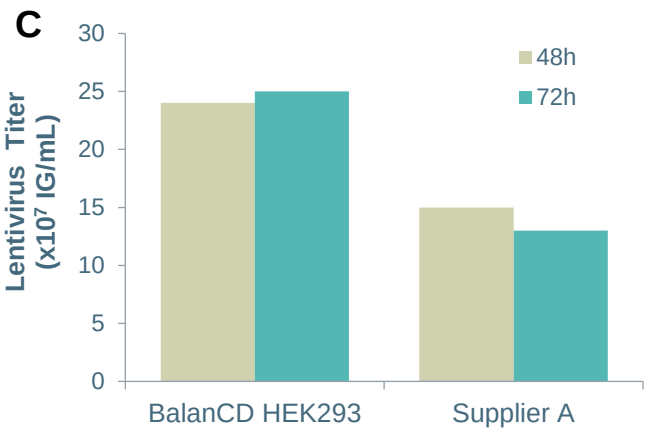
Lentivirus Production

CULTURE CONDITIONS USED IN THIS EXAMPLE

- Medium: BalanCD HEK293 supplemented with 4mM L-Glutamine and 0.1% Poloxamer
- Cell stock: HEK293T cells directly thawed into BalanCD HEK293 medium, supplemented as stated above, and passaged every 3 to 4 days for three passages before going into the bioreactor production run
- Culture vessel: Benchtop Bioreactor with 2L working volume
- Seeding density: 1.5 x 10^5 cells/mL
- Incubator setting: 37°C, 5% CO_2, humidified
- Transfection on day 3
    - >90% viability
    - Transfection agent: PEI
    - 4 plasmids for Lentivirus
    - DNA:PEI ratio at 1:1.5
    - Total amount of 2.5 µg DNA per 10^6 cell
    - Cell culture was supplemented with Sodium Butyrate at 24h post transfection







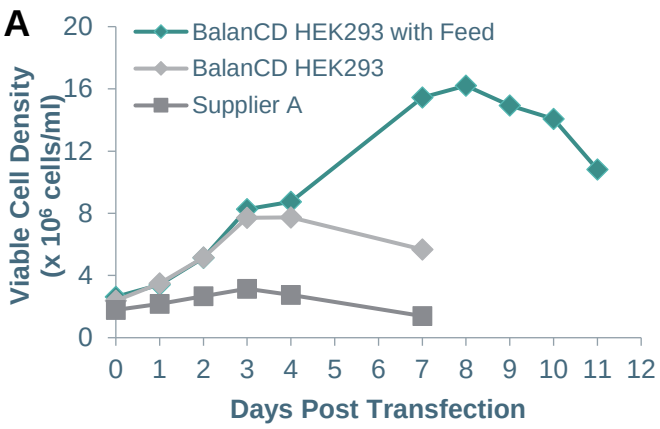
BalanCD HEK293 supports cell growth and lentiviral vector production. Viable cell density **(A)** and percent viability **(B)** were determined. Lentivirus titer was measured at 48 and 72 hours post transfection **(C)**. Culture supernatants were treated with DNase and serially diluted before inoculation on HCT116 cell monolayers. One day later, the cells were trypsinized and lysed for DNA extraction. The integrated vector genome are quanitfied by qPCR.

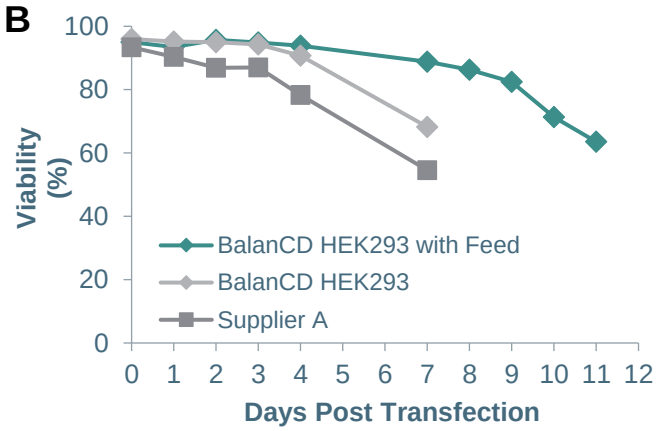
Transient Protein Expression

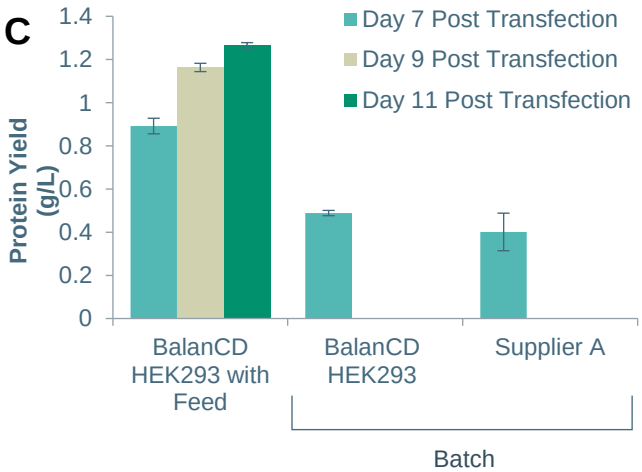
Since optimal transfection parameters may vary based on a number of factors including, cell type, method of transfection (PEI, lipofection, electroporation, etc.), and DNA source, optimization of parameters is highly encouraged. The following PEI-mediated transient transfection protocol can be used as a general guideline to begin optimization work.

CULTURE CONDITIONS USED IN THIS EXAMPLE

- Cells: HEK293-6E cells (Canadian National Research Council)
- Medium: BalanCD HEK293 supplemented with 4 mM L-Gln
- Culture vessels: Corning 125 mL Baffled Polycarbonate shake flask
- Working Volume: ~20-25% of flask volume (i.e. 25-30 mL in a 125 mL flask)
- Seeding density: 3 x 10^5 cells/mL
- Rotation speed: 140 rpm
- Incubator: 37°C, 5% CO_2, humidified
- Transfection 3 days post seeding
    - VCD at 1.5-2.0 x 10^6 cells/mL with >90% viability
    - Transfection agent: PEI
    - Hc, Lc of Biosimilar antibody, AKT, and GFP at 40%,40%,15% and 5%
    - DNA:PEI ratio at 1:3
    - Total amount of 1 µg DNA per 10^6 cells
- **BalanCD HEK293 Feed on Days 1-4 Post-Transfection**
    - BalanCD HEK293 Feed was added at 5% working volume each day on days 1-4 post-transfection.
        - 1.5 mL of BalanCD HEK293 Feed was added for 30 mL culture at each feeding event.
    - Optional: 2 mL/L of Anti-Clumping Supplement may be added if cells start to aggregate as higher VCD is achieved.
      It was not necessary to add the supplement to the cultures used in this example, as cell aggregation was not observed.





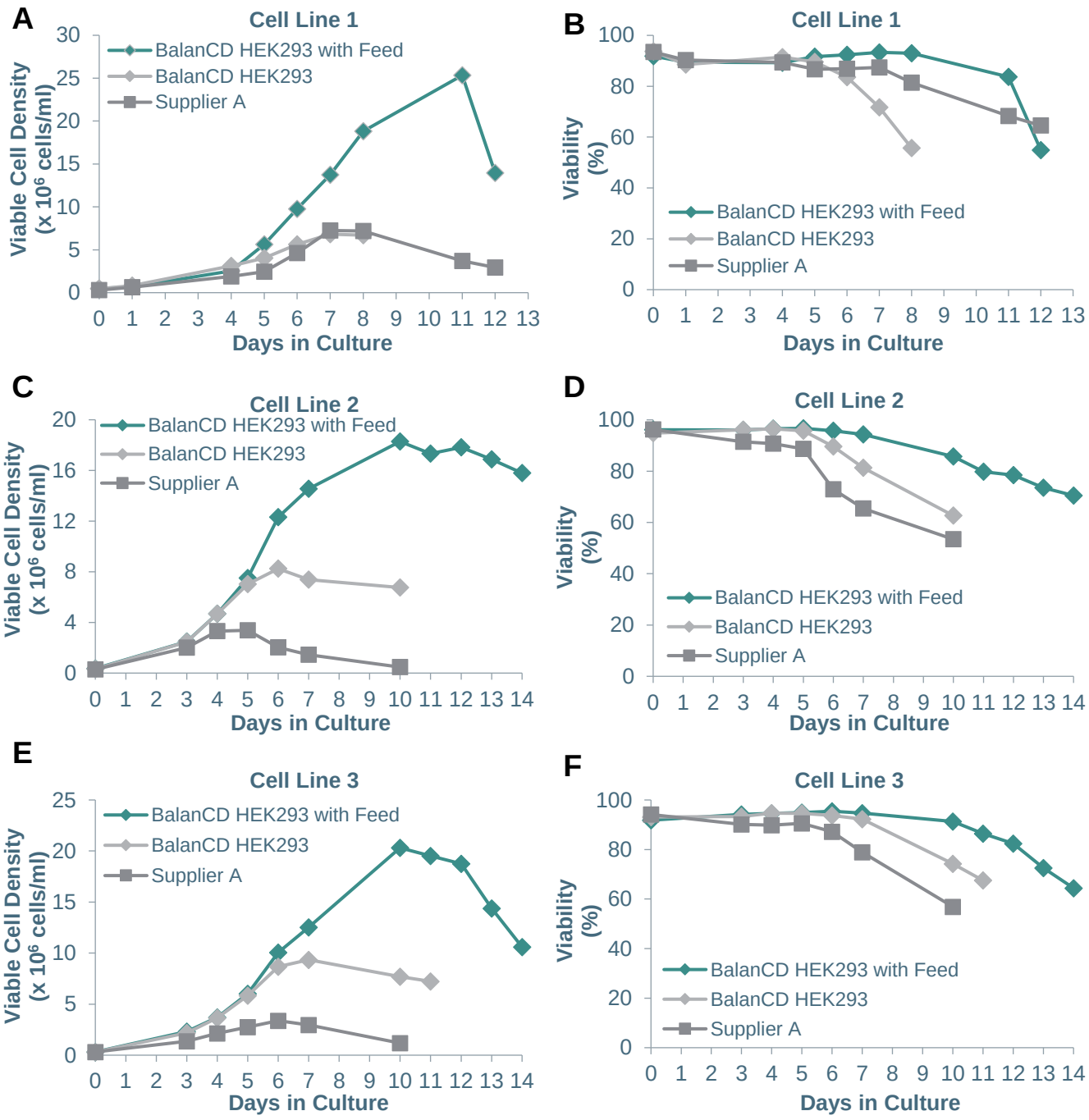


BalanCD HEK293 system supports high cell density, culture longevity, and increased yield. Viable cell density **(A)** and percent viability **(B)** were measured. On day 1 post-transfection, transfection efficiency (approx. 40%, data not shown) was measured utilizing flow cytometry. BalanCD HEK293 was supplemented with a 5% v/v addition of BalanCD HEK293 Feed on days 1-4 post-transfection. Protein titer **(C)** was measured on days 7, 9, and 11 post-transfection The batch conditions were terminated on day 7 due to a viability <70%. Data shown represents the average of two duplicate cultures.

High Density Culture

The BalanCD HEK293 system of media and supplement enables high viable cell density of a variety of HEK293 cell lines. Three different commercially available HEK293 cell lines were cultured in Corning 125 mL baffled polycarbonate shake flask, at a working volume of 20-25% of flask volume (i.e. 25-30 mL in a 125 mL flask), and seeding density of 3 x 10^5 cells/mL in a 37°C, 5% CO_2, humidified incubator. The rotation speed was set at 140 rpm.

BalanCD HEK293 medium was supplemented with 2 mL/L of Anti-Clumping Supplement and 4 mM L-Gln.
4% v/v of BalanCD HEK293 Feed was added to cultures on days 3-7 post inoculation.



BalanCD HEK293 System supports high density and culture longevity for multiple HEK293 cell lines. Viable cell density **(A, C, E)** and percent viability **(B, D, F)** were measured for the three HEK293 cell lines. Data shown represents the average of two duplicate cultures. Cell line 1: HEK293.2sus (ATCC); Cell line 2: Expi293F™ (Thermo Fisher Scientific); Cell line 3: 293-F (Thermo Fisher Scientific).

Related Products

Catalog #	Product	Size
91166	BalanCD HEK293 Feed	500 mL
91150	Anti-Clumping Supplement	50 mL
9317	L-Glutamine Solution (200mM)	100 mL, 500 mL
9240	1X PBS, Dulbecco's Phosphate Buffered Saline	100 mL, 500 mL, 1L

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: tmrequest@irvinesci.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

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

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

FUJIFILM Irvine Scientific, Inc.
2511 Daimler Street, Santa Ana, California 92705 USA
Telephone: 1 949 261 7800 • 1 800 437 5706
Fax: 1 949 261 6522 • www.irvinesci.com

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