BalanCD HEK293 Perfusion A

Catalog #	Product	Format	Available Package Sizes*
91184	BalanCD HEK293 Perfusion A	Liquid	1 L bottle, 20 L bag
94155	BalanCD HEK293 Perfusion A	Powder	10 L

*Additional package sizes are available upon request.

Intended Use

For research or further manufacturing use only.

Product Description

BalanCD HEK293 Perfusion A is a chemically defined, animal component-free growth medium for Human Embryonic Kidney 293 (HEK293) cells specifically designed for growth and production of viral vectors in the perfusion process. BalanCD HEK293 Perfusion A is provided without L-Glutamine to extend shelf life. The recommended L-Glutamine concentration to add is 4-8 mM (20-40 mL/L). Glucose and glutamine should be monitored during perfusion culture, as additional supplementation may be required. BalanCD HEK293 Perfusion A has been designed to support various HEK293 cell lines growth and productivity in perfusion-capable bioreactors with a perfusion rate as low as 25 pL/cell/day with minimal buildup of undesirable metabolites such as lactate and ammonia. The medium does not contain antibiotics, antimycotics, protein hydrolysates, or any other undefined components. The product contains no hydrolysates, L-Glutamine, antibiotics, antimycotics, or any other undefined components, and is ready to use for suspension culture applications. It can be used with the Anti-Clumping Supplement (Catalog # 91150) 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 https://fujifilmbiosciences.fujifilm.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. Avoid prolonged exposure to light or elevated temperatures. This product is stable for 12 months, when unopened and stored properly. Do not use it after the assigned expiration date. Not validated for use beyond the unopened expiry shelf life. Do not use any bottle or bag 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 and allow the powder to settle for a few minutes before opening and reseal tightly after use. The powder should be free flowing; do not use it if it is caked. This product is stable for 24 months, when unopened and stored properly. Avoid opening and closing the container repeatedly, as exposure to air increases the risk of moisture absorption. Do not use it after the assigned expiration date.

Directions for Use

HYDRATION OF BALANCD HEK293 PERFUSION A FROM POWDER MEDIUM

1. Add powder medium (28.12 g/L, Catalog # 94155) to WFI (971 mL/L, Catalog # 9309 or equivalent) into an appropriately sized container.
2. Mix the solution at 300-350 rpm for approximately 10 minutes or until the powder is well dissolved (the solution may still appear cloudy at this point). Use medium speed to avoid any aeration or bubble forming.
3. Add 2.20 g/L Sodium Bicarbonate to the solution and mix at moderate speed until completely dissolved.
4. The medium is formulated to meet specific pH and osmolality requirements—pH 6.9–7.4 and osmolality 280–320 mOsm/kg**.** However, if these values fall outside the expected range due to any factor during preparation**,** adjust as needed**.** It is recommended to use 5N HCl or NaOH to bring the pH within specification.
5. Sterile filter through a 0.2 or 0.1 µm PES membrane filter.
6. The solution can be stored in the dark at 2-8°C for up to 1 year.
7. Supplement 20 mL/L or 40 mL/L of 200 mM L-Glutamine (Catalog # 9317) to BalanCD HEK293 Perfusion A medium to reach 4 mM or 8 mM final concentration prior to use (depends on application).

FOR BIOREACTOR USE:

1. If required, filter the media through a 0.2 or 0.1 µm filter into a validated sterile bag.
2. Aseptically add L-Glutamine to the final concentration of 8 mM.
3. Aseptically connect the media bag to the bioreactor feed line using sterile welding or Luer connections.
4. Prime the tubing and remove air bubbles by allowing media to flow through the line.
5. Fill the bioreactor to the desired working volume.
6. Adjust bioreactor settings to the desired temperature, pH, dissolved oxygen (DO), and agitation rate.
7. Complete a 24-hour sterility hold to ensure sterility in the bioreactor; during this time, the cell culture media will be conditioned to the desired set points.
8. After 24 hour sterility hold, complete a pH/DO offset step if needed before inoculating cells into bioreactor
9. Inoculate cells into bioreactor.
10. Monitor pH, osmolality, and metabolite levels regularly, and supplement culture with glucose and glutamine, if required. If glucose levels drop below 3 g/L, supplement in a single bolus to restore levels to 6 g/L. To prevent glucose depletion, administer an additional 2 g/L incrementally over 24 hours using a pump.

    Note: This feeding strategy can be repeated multiple times per day as needed. However, glucose concentration must not exceed 6 g/L and should be maintained ideally between 3-6 g/L for optimal cell growth.

CELL RECOVERY AND ADAPTATION

1. Supplement BalanCD HEK293 Perfusion A medium (Catalog # 91184 or 94155) with 4 mM L-Glutamine (Catalog # 9317). Aseptically transfer appropriate volume (30 mL) of supplemented medium into a 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 Perfusion A 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 Perfusion A. After a minimum of 3 passages, 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 Perfusion A.

SUB-CULTURING PROCEDURE

1. Supplement BalanCD HEK293 Perfusion A medium with 4 mM of L-Glutamine.
2. Sub-culture cell stocks every 3 to 4 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.
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.

    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. Freshly prepare required volume of freezing medium (90% cold BalanCD HEK293 Perfusion A + 10% DMSO). Keep at 4°C until ready to use.
2. Centrifuge appropriate number of healthy cells for 5 minutes at 200 g 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.
7. Thaw the frozen vial rapidly in a 37°C water bath and transfer the cell suspension to a culture flask with pre-warmed BalanCD HEK293 Perfusion A medium to achieve an initial cell density of 3 x10^5 cells/mL. Incubate cultures at 37 °C and 5% CO2 for 3-4 days.
8. Sub-culture cells following the sub-culture procedure. Cells usually grow slowly in the first 1-2 passages. A minimum of 3 passages in BalanCD HEK293 Perfusion A is strongly recommended before use.

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: fisitmrequest@fujifilm.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

Visit https://fujifilmbiosciences.fujifilm.com/ for technical resources and information including:

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

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P/N 021515 Rev. 02
Effective Date: 19 Nov 2025