Growth and Maintenance of BHK Cells

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Culturing BHK Cells

Shipping/Storage

Cells are shipped on dry ice. Store in liquid nitrogen upon receipt.

Contents

One vial contains 3 x 10^6 baby hamster kidney (BHK) cells in 1 ml of αMEM, 10% fetal bovine serum, 10% DMSO.

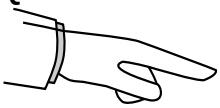
BHK Cells

The BHK cell line was derived from baby Syrian hamster (*Mesocricetus auratus*) kidney (Macpherson and Stoker, 1962).

- The medium for BHK cells is αMEM or DMEM (BioWhittaker).
- Complete medium for BHK cells is αMEM + 2 mM L-glutamine + **5% fetal bovine serum**. FBS does not need to be heat inactivated for use with BHK cells. **Note: 10% FBS makes the cells grow too fast. Infection with Sindbis viral particles will result in premature cell lysis, leading to low yields of protein expression.**
- Cells are grown in a humidified, 37°C, 5% CO_2 incubator.
- If cells are split at 1:5, it will take 1-2 days to reach 80-90% confluency.

Product Qualification

BHK cells must be 85% viable when recovered in αMEM medium. The cells are thoroughly tested for the absence of mycoplasma contamination.



Note

The cells have a tendency to clump in complete medium. In general, this is not a problem except when preparing the cells for electroporation. In this case, care must be taken to avoid clumps. PBS is required to keep the cells from clumping during electroporation.

General Cell Handling

Use the procedures below to initiate and maintain a culture of BHK cells.

- **All solutions and equipment that come in contact with the cells must be sterile.**
- Always use proper sterile technique and work in a laminar flow hood.
- Use cells that are 80-90% confluent and > 90% viability for transfections and infections.
- Before starting experiments, be sure to have cells established and also have some frozen stocks on hand.
- For general maintenance of cells, pass BHK cells when they are 80-90% confluent (1-2 days) and split at a 1:5 dilution. For example, transfer 2 ml of a 10 ml cell suspension (without trypsin/EDTA) to a new 75 cm^2 flask and add 10 ml fresh medium.
- Use trypan blue exclusion to determine cell viability. Thawed cells should be 80 to 85% viable and healthy, log phase cultures should be > 90% viable.
- Cells may be passaged 60-70 times before re-starting a culture from frozen stocks.

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Culturing BHK Cells, continued

Before Starting

Be sure to have the following solutions and supplies available:

- 15 ml sterile, conical tubes
- 5, 10, and 25 ml sterile pipettes
- Cryovials
- PBS (page 5)
- 0.4% Trypan blue in PBS
- Hemacytometer
- αMEM medium (BioWhittaker)
- Tissue culture grade 200 mM L-glutamine
- FBS
- Complete αMEM medium (αMEM + 2 mM L-glutamine + 5% FBS, page 5)
- Freezing Medium (αMEM medium + 2 mM L-glutamine + 10% FBS + 10% DMSO, page 5)
- Table-top centrifuge
- 75 cm^2 flasks, 175 cm^2 flasks and 35 mm plates (other flasks and plates may be used)
- Trypsin/versene (EDTA) solution (BioWhittaker) or other trypsin solution

Initiating Cell Culture from Frozen Stock

The following protocol is designed to help you initiate a cell culture from a frozen stock. Note that the vial of BHK cells contains 3 x 10^6 cells.

1. Remove the vial of cells from the liquid nitrogen and thaw quickly at 37°C.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol, and transfer the cells to a 15 ml sterile, conical tube.
3. Add 9 ml of prewarmed (37°C), complete αMEM medium dropwise to cells.
4. Centrifuge in a table-top centrifuge at 250 x g for 5 minutes at room temperature. Decant the medium. (This removes the DMSO from the cells.)
5. Resuspend the cells in 10 ml of complete αMEM and test a small portion of the cell suspension for viability by trypan blue dye exclusion. Viability of the cells should be between 80 and 85%.
6. Transfer the remaining cell suspension to a 75 cm^2 flask, and incubate at 37°C. Incubation for 1-2 days should yield an 80-90% confluent monolayer.

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Culturing BHK Cells, continued

Passaging the BHK Cells

1. When cells are ~80-90% confluent, remove all medium from the flask.
2. Wash cells once with 10 ml PBS to remove medium. Serum contains inhibitors of trypsin.
3. Add 5 ml of trypsin/versene (EDTA) solution to the monolayer and incubate 1 to 5 minutes at room temperature until cells detach. Check the cells under a microscope and confirm that most of the cells have detached. If cells are still attached, incubate a little longer until most of the cells have detached.
4. Once the cells have detached, briefly pipet the solution up and down to break up clumps of cells.
5. Add 5 ml of complete αMEM to stop trypsinization. Centrifuge cells at 250 x g for 5 minutes.
6. Aspirate the supernatant and resuspend the cells in 10 ml complete αMEM. Check the viability of the cells. Cells should be > 90% viable.
7. To maintain cells in 75 cm^2 flasks, transfer 2 ml of the 10 ml cell suspension from Step 6 to a new 75 cm^2 flask and add 10 ml fresh, complete αMEM medium.
8. To expand cells, transfer 3 to 4 ml of the cell suspension to a 175 cm^2 flask (3 flasks total) and add fresh, complete αMEM medium to a final volume of 30 ml.
9. Incubate flasks in a humidified, 37°C, 5% CO_2 incubator.

Repeat Steps 1-9 as necessary to maintain or expand cells.

Freezing the BHK Cells

Before starting, label cryovials and prepare freezing medium (page 5).

1. When cells are ~80% confluent in a 175 cm^2 flask, remove the medium and wash the cells one time with 10 ml PBS.
2. Add 5 ml of trypsin/versene (EDTA) solution and incubate 1 to 5 minutes until cells detach. Once cells have detached, briefly pipet solution up and down to break up clumps of cells.
3. Add 5 ml of complete αMEM to stop trypsinization. Count the cells in a hemacytometer.
4. Pellet cells at 250 x g for 5 minutes in a table top centrifuge at +4°C and decant the medium.
5. Resuspend the cells at a density of 3 $x10^6$ cells/ml in freezing medium (see page 5).
6. Aliquot 1 ml of the cell suspension per vial. Place vials at -20°C for 2-3 hours.
7. Transfer vials to a -70 or -80°C freezer and hold overnight.
8. Transfer vials to liquid nitrogen for long term storage.

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Culturing BHK Cells, continued

Complete αMEM Medium

To 500 ml αMEM (BioWhittaker), add 5% FBS and 5 ml of 200 mM L-glutamine solution (BioWhittaker).

Freezing Medium

αMEM containing 2 mM L-glutamine, 10% FBS, and 10% DMSO.

Phosphate Buffered Saline

For washing cells only. The solution does not need to be RNase-free.

137 mM NaCl
2.7 mM KCl
10 mM Na_2HPO_4
1.8 mM KH_2PO_4

1. Dissolve: 8 g NaCl
   0.2 g KCl
   1.44 g Na_2HPO_4
   0.24 g KH_2PO_4

   in 800 ml deionized water.
2. Adjust pH to 7.4 with concentrated HCl.
3. Bring the volume to 1 liter and autoclave for 20 minutes on liquid cycle.
4. Store at +4°C or room temperature.

Reference

Macpherson, I. A. and Stoker, M. G. P. (1962) *Virology*, **16**: 147.

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