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Innovative Life Science Solutions™

Product Description ©2016

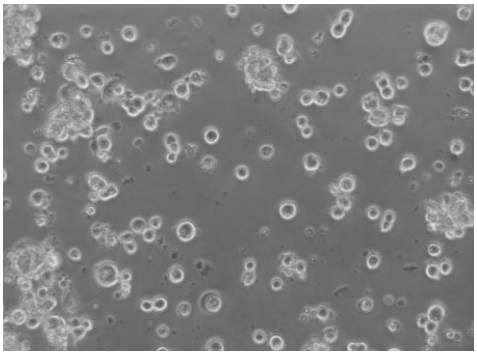
IBHK-4, A PROPRIETARY BHK DERIVED CELL LINE

General Description and Source

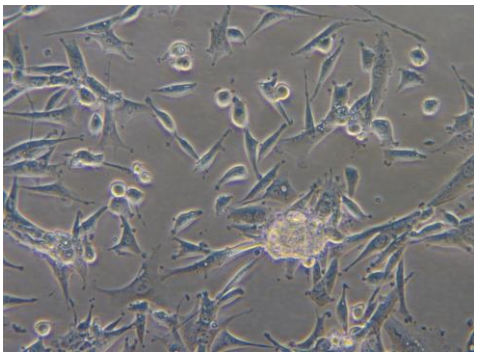
The IBHK-4 cell line, which is routinely grown in INCELL's ACE™ medium **(Cat num: AACE-500; AACE-1000)** as a suspension culture, was originally derived by INCELL as a single cell cloned population "clone 4" from the BHK21-C13 cell line (1). The IBHK-4 cell line has been verified as being of hamster origin by both isoenzyme and species-specific antibody analyses. Addition of serum or sets of specific growth and attachment factors to ACE™ medium followed by seeding cells onto a monolayer substrate stimulates re-growth of monolayer cultures of the same general size and appearance as the parental cell line, but with a larger number of rounded and aggregate cells such that a mixed suspension-monolayer culture is maintained.

The IBHK-4 cell line was selected and adapted for growth under serum-free, antibiotic-free and animal product-free conditions in ACE™ culture medium (INCELL) in stirred bioreactors. The suspension culture derived cells efficiently form colonies in soft agar and, as expected, are tumorigenic in hamsters. The IBHK-4 cell line has been used as a permissive cell line to culture viruses, such as the vaccinia virus strain MVA (Modified Vaccinia Ankara), which is intended for vaccine production for poxvirus protection or as a recombinant vector.

Figure 1. IBHK-4 Cells Growing in Suspension Culture



Healthy cells grow in aggregates and as single cells in suspension (ACE™ culture medium, phase contrast x200)



IBHK-4 cells transferred to serum-supplemented ACE™ culture media are a mixed monolayer-suspension culture similar to parental BHK21-C13 cells (phase contrast x200)

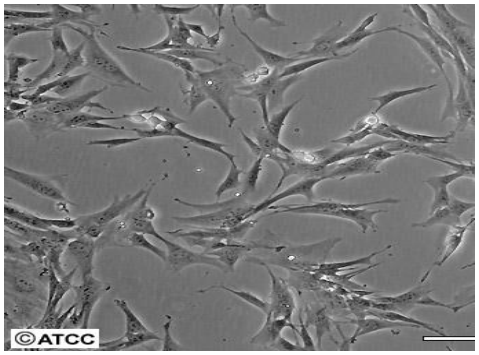


Figure from ATCC website of parental BHK-21C13 cell line. Bar=100microns

Reconstitution from Cryovials into Stirred Bioreactor Culture Vessels or Flasks

Thaw cryovial(s) rapidly in a 37°C water bath. For maximum viability, the whole process should be completed within 30 minutes. Gently and aseptically transfer cells to 2 ml warmed culture medium in a 15 ml conical centrifuge tube. Add about 4 ml of additional growth medium, pellet the cells by centrifugation, then add 10 ml fresh, complete growth medium and re-suspend the cells. Transfer cell suspensions of about 2 x 10^5 viable cells/mL to each culture vessel or flask and incubate at 37°C in 5% CO_2 and air.

Propagation and Subculture

The ACE™ serum-free, animal product, and antibiotic-free growth medium is used for normal maintenance of cells, which are sensitive to relative culture density. IBHK-4 cells in suspension, in a stirred bioreactor, should be kept at densities between 1.7 x 10^5 cells/mL to 1x10^6 cells/mL. If the culture is allowed to grow to high density, the cells will lyse and cell debris may accumulate.

Standard cell counting methods are used to determine cell numbers, seeding and culture density. Cell populations may be subcultured any time they are denser than $2x10^5$ cells/mL, but must be subcultured once they reach a density of about $1x10^6$ cells/mL. The recommended seeding density is 2×10^5 cells/mL for a two-day maintenance schedule or 1.7×10^5 cells/mL for a three-day maintenance schedule.

For bioreactors, calculate the number of cells and reseed bioreactor with added fresh complete culture medium or use cells to expand and seed additional flasks or bioreactor cultures. Not more than 25% (v/v) conditioned medium should be carried over upon subculture. Cells may be centrifuged and seeded completely in fresh growth medium. If it is necessary, a density as low as 1×10^5 cells/mL may be seeded but 25% conditioned medium is recommended.

Incubators are to be maintained at 37°C, but no CO_2 is required for ACE™-medium cultured cells.

Cryopreservation

Cells are cryopreserved in INCELL's ready-to-use **EZ-CPZ™ (Cat num: EZCZ-50, EZCZ-100)** using standard methods of slow-freeze and rapid thaw for re-animation. Freeze actively growing cells at $1x10^7$ cells/mL in 4 mL cryo vials as 1:1 conditioned medium: EZ-CPZ™. Each cryo vial will contain $4x10^7$ cells, 2 mL conditioned medium and 2 mL EZ-CPZ™. Alternatively, cells may be frozen at a density of $1x10^7$ cells in 1.5 mL total volume of 1:1 conditioned media:EZ-CPZ™. Storage temperature: Liquid nitrogen vapor phase.

Biohazard and Infectious Agent Considerations

These cells were derived in the INCELL laboratories from the parental BHK21 Clone 13 cell line They are believed to be free of all known infectious agents. They are negative in tests of hamster associated pathogens (HAP) tests and do not release infectious retrovirus. Endotoxin by Limulus amebocyte lysis gel clot LAL assay has been negative and standard sterility tests for bacterial, fungal and mycoplasma have been negative. Additional vaccine cell substrate testing is in progress.

Cell Line Distribution

Cells are not sold but are distributed by INCELL with a Cell Licensing Agreement and Material transfer Agreement (CLMTA). The CLMTA form can be requested by phone (toll-free 800-364-1765; or directly 210-877-0100), by email to info@incell.com, or by FAX 210-877-0200.

REFERENCE

(1) The parent line of BHK-21clone13 (ATCC CCL-10) was derived in 1961 from the kidneys of five unsexed, 1-day-old Syrian golden hamsters (*Mesocricetus auratus*) by I. Macpherson and M. Stoker (Virology 16: 147-151, 1962). After 84 days of continuous cultivation, interrupted only by cryopreservation over an 8-day period, clone 13 was initiated by single-cell isolation (Macpherson I. J. Natl. Cancer Inst. 30: 795-815, 1963). BHK-21(C-13) has been used extensively to study cell biology and as a high quality substrate for viruses, including many veterinary vaccines, and other cell-associated infectious agents. The cell line is also used as a substrate for the production of recombinant human factor VIII (i.e., Kogenate; Bayer).