Cell Line Designation: SK-BR-3
AddexBio Catalog No. C0006007

Cell Line Description:

Origin: Breast adenocarcinoma.
Species: Homo sapiens
Tissue: Breast; mammary gland
Properties: Mixed adherent and loosely epithelial, with rounded, some floating (see remarks)
Cytogenic data: This is a hypertriploid to hypotetraploid human cell line(+A, +B, +C, +E, +F, +G, -D) with abnormalities including dicentrics, acrocentric fragments, rings, secondary constrictions, large metacentrics or polycentrics and large submetacentric marker
Patient: Female, Caucasian, 43 yrs of age
Complete Medium: AddexBio-Formulated RPMI-1640 Medium (C0004-01) or McCoy's 5a Medium + 10% FBS
Subculture Procedure: 1:2 using 0.25% trypsin or trypsin/EDTA, 5% CO2; 37°C
Medium Renewal: Two to three times weekly.
Freezing Medium: Complete culture medium supplemented with 5% (v/v) DMSO
Additional Information: Additional product and technical information can be obtained from the catalog references and the Addexbio Technical Information site at www.addexbio.com, or by email at customersupport@addexbio.com.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafty/bmbl4/bmbl4toc.htm

Use Restrictions: **These cells are distributed for research purposes only.** Addexbio does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Cells Upon Arrival:

Frozen cells must be thawed immediately upon receipt and grown according to the handling procedures described here in this instruction manual to ensure the best cell viability.

Note: ***Avoid refreezing or repetitive freezing cells upon receipt*** *as it may result in irreversible damage to the cell line.*

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AddexBio Technologies
4907 Morena Blvd, Ste 1408
San Diego, CA 92117 USA

858-348-7819
Fax: 858-538-8847
Email:customersupport@addexbio.com
www.addexbio.com

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures described in this instruction manual.

Handling Procedure for Frozen Cells:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

Safety Precaution:

Addexbio highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer and resuspend the vial contents to a T25 flask with recommended complete medium. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).
4. One may also transfer the vial contents into a new culture flask if removal of DMSO is not important. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).
5. Incubate the culture at 37°C in a suitable incubator for 24-48 hours for cell attachment. A 5% CO_2 in air atmosphere is recommended.

Handling Procedure for Cells in Flask Culture:

The flask was seeded with cells grown and completely filled with complete medium at AddexBio facility that acts as a cushion and to prevent loss of cells during shipping.

1. Upon receipt, carefully examine if the majority of the cells are attached to the bottom of the flask using an inverted microscope (preferably equipped with phase-contrast optics),

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as the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable) during shipping. In addition, visually examine the culture for macroscopic evidence of any microbial contamination.

2. **For the cells are still attached**, asceptically remove all but 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **For the portion of cells that are not attached**, asceptically remove the entire contents of the flask but 10 ml of the shipping medium and centrifuge at 125 x g for 5 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to the same 25 cm^2 flask (T25). Incubate at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
4. Cells will need some time to recover from the shipping journey. Keep watching the cells and replace medium (10 ml) every two days without disturbing the monolayer for the first week or until they are 80-85% confluent.

Cell Subculture:

1. Remove and discard culture medium in the flask
2. Gently rinse the cell layer with 0.25% (w/v) Trypsin, 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 1.0 mL (T25) to 2 mL (T75) of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 min). Avoid tapping the flask at this stage so that cells are not likely to clump.
4. Add 10 mL of complete medium and aspirate cells by gently pipeting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C/5% CO2.

Subcultivation Ratio: 1:2; **Medium Renewal**: 2-3 times per week.

References for SK-BR-3 cells:

1. Brockhoff, G., Heckel, B., Schmidt-Bruecken, E., Plander, M., Hofstaedter, F., Vollmann, A., and Diermeier, S. Differential impact of Cetuximab, Pertuzumab and Trastuzumab on BT474 and SK-BR-3 breast cancer cell proliferation. Cell Prolif, 40: 488-507, 2007.

2. Choi, E. J. and Kim, G. H. Apigenin causes G(2)/M arrest associated with the modulation of p21(Cip1) and Cdc2 and activates p53-dependent apoptosis pathway in human breast cancer SK-BR-3 cells. J Nutr Biochem, 20: 285-290, 2009.

3. Hill, A. A., LaPan, P., Li, Y., and Haney, S. Impact of image segmentation on high-content screening data quality for SK-BR-3 cells. BMC Bioinformatics, 8: 340, 2007.

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Lot Specific Information Sheet for AddexBio Cat #: C0006007

Lot#: 0033160

Designation: SK-BR-3 cells

Total Cells/mL: >1x10^6

Expected Viability: 75.0-81.2%

Ampule Passage #: 11

Dilute Ampule Content: 1:10 (T-25)

Volume/Ampule: 1 mL

A T-25 setup at a dilution of 1:10, using culture medium as described in the product information sheet, reaches approximately 25-42% confluence within 24 hours.

Remarks:

Attachment of SK-BR-3 cells will be light during initial recovery from cryopreservation. This is normal. It is not uncommon for this cell line to show loose attachment and floating cells during the entire first week in culture and also after each subculture. The adherent cells will show an epithelial-like morphology or a rounded morphology. Some cells will have a flattened, cobblestone appearance, and other cells remain rounded but loosely attached. If the culture is allowed to overgrow, the cells may pile and detach from the flask. Some cell debris will typically also be present. Floating cells should always be retained by gentle centrifugation (125 x g for 5 to 7 minutes) and added back to the adherent population when feeding with fresh medium every 2-3 days.

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