Cell Line Designation: AU565 [AU-565]

AddexBio Catalog No. C0006010

Cell Line Description:

Disease: Adenocarcinoma

Origin: The AU565 cell line was derived from a pleural effusion of a patient with breast carcinoma.

Species: Homo sapiens, human

Tissue: Breast, mammary gland; derived from metastatic site: malignant pleural effusion

Properties: Epithelial, adherent

Complete Medium: Formulated RPMI-1640 Medium (C0004-01)[compare formulation before culturing if not using our medium C0004-01, please see remarks below] + 10% FBS

Subculture Procedure: Note: DO NOT allow cells to become confluent. Subculture at 50-60% confluency. The majority of cells should be round and undefferentiated. If cells overgrow, they can be brought back to an undifferentiated condition by maintaining them under sparse conditions for a couple of passages. Subculture at 1:2 -1:4 using trypsin/EDTA; culture at 5% CO_2, 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.

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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.*

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 the vial contents to the centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125xg for 5 to 7 minutes.

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4. Resuspend cell pellet with the recommended complete medium and dispense into a new culture flask. 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).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended.

References for AU565 cells:

1. Bacus SS, et al. Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen. Mol. Carcinog. 3: 350-362, 1990.

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Lot Specific Information Sheet for AddexBio Cat #: C0006010

Lot Number: 0143922

Designation: AU565 CELLS

Total Cells/mL: >$1.2x10^6$

Expected Viability: 80.0-85.0%

Ampule Passage #: 16

Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)

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 50% confluence within 3 days.

Remarks:

- Do not carry out viability test the time when the cells are thawed. The result is not accurate.
- Do not culture this cells with anti-fungal reagents that will slow down the growth.
- Please clearly read this instruction manual and compare the medium formulation before proceeding.

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