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| Code: | 0363 |
| Cell Line: | AN3 CA |
| Species: | Homo sapiens Vulgar Name: Human |
| Tissue: | Uterus/endometrium |
| Morphology: | Epithelial |
| Disease: | Adenocarcinoma |
| Growth Properties: | Adherent |
| Sex: | FEMALE |
| Age Ethnicity: | 55 years; Caucasian |
| Tumor Formation: | Yes, in nude mice. Yes, in the cheek pouch of cortisone treated hamsters. |
| Biosafety: | 1 |
| Culture Medium: | Dulbecco's Modified Eagle's Medium (DMEM) with 1% non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate + 10% FETAL BOVINE SERUM. |

Subculturing:

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 1.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Place culture vessels in incubators at 37°C. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

Medium Renewal: 2 to 3 times per week

Subcultivation ratio: 1:3 to 1:6 is recommended.

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

**Thawing Frozen
Cells:**

SAFETY PRECAUTION: It is highly recommended 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 submerged 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 18-20°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the 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. For cells that are sensitive to DMSO it is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio).
5. Incubate the culture in an appropriate atmosphere and temperature (see "Culture Conditions" for this cell line).

References:

Dawe CJ, et al. Growth in continuous culture, and in hamsters, of cells from a neoplasma associated with Acanthosis nigricans. *J. Natl. Cancer Inst.* 33: 441-456, 1964. PubMed: 14207855

Goodfellow M, et al. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. *J. Natl. Cancer Inst.* 59: 221-226, 1977. PubMed: 77210034

Hendricks DT, et al. FHIT gene expression in human ovarian, endometrial, and cervical cancer cell lines. *Cancer Res.* 57: 2112-2115, 1997. PubMed: 9187105

The cells produce undifferentiated malignant tumors. at low frequency (22%)

C. J. Dawe and associates derived this cell line from a metastatic lesion in the lymph node of a patient with endometrial carcinoma alerted to the condition by onset of the malignant disorder acanthosis nigricans.

Depositors:

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ATCC:

HTB-111