BCRJ 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/Ethinicity:	55 Year / 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 2 mM L-glutamine and 10% of 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.
Subculturing Medium Renewal:	2 to 3 times per week
Subculturing 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 strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris. 1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions. 3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes. 4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio). 5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).
References:	Dawe CJ, et al. Growth in continuous culture, and in hamsters, of cells from a neoplasma assoicated 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.
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