Product sheet

A431 | 300112

General information

Description	A-431 cells are a human cell line derived from a solid epidermoid carcinoma tumor in an 85-year-old female patient. This cell line has an epithelial morphology and grows in clusters. Because of their high EGFR expression levels are frequently used as a positive control for EGFR expression in cancer, toxicity, and immuno-oncology studies. A-431 cells stimulated by EGF undergo rapid tyrosine phosphorylation of intracellular signaling proteins that control cellular processes such as growth, proliferation, and death. EGF promotes A-431 cell proliferation at low concentrations, but at higher concentrations, it inhibits cell growth by inducing terminal differentiation. Additional in vitro studies show that EGF significantly inhibits protein synthesis and DNA replication. Although several receptors are involved in the EGF-induced down-regulation of A431 cells, the loss of EGFR expression is less pronounced than in other cell types. Bradykinin inhibits both baseline and EGF-induced EGFR phosphorylation in A-431 cells. In response to phorbol esters and Sertoli cell-secreted growth factor (SCSGF), A-431 cells express the interleukin 1-related protein IL1H. Because of their high EGFR expression, A-431 cells are frequently used in cancer-related studies on the cell cycle and cell signaling pathways. They are highly susceptible to mitogenic stimulation because they lack a functional copy of p53, a potent tumor suppressor gene. A-431 cells demonstrated antitumorigenic effects of additional EGF and radiation-sensitive characteristics in xenografts. The A-431 cell line is an appropriate cell model for evaluating cancer therapy because it has been engineered to express tumor antigens such as mesothelin and GPC3. A-431 cells are hypertriploid with a median chromosomal number of 74, which is found in 36% of them. They can proliferate in soft agar colonies and subcutaneous tumors in mice with compromised immunity. The cells express many isoenzymes, including AK-1, ES-D, G6PD, GLO-I, Me-2, PGM1 and PGM3.
Organism	Human
Tissue	Epidermoid
Disease	Squamous cell carcinoma
Synonyms	A-431, A431/P

Characteristics

Age	85 years
Gender	Female
Morphology	Epithelial-like, flat polygonal
Growth properties	Adherent

Identifiers / Biosafety / Citation

Citation	A431 (Cytion catalog number 300112)

Biosafety level	1

Expression / Mutation

Receptors expressed	EGF-binding sites
Protein expression	p53 positive
Isoenzymes	G6PD, B, PGM1, 1, PGM3, 1, ES-D, 1, Me-2, 0, AK-1, 1, GLO-1, 2
Tumorigenic	Yes, in immunosuppressed mice
Products	HBp17
Mutational profile	BRAF V600Ewt
Karyotype	Six marker chromosomes with rearrangements: der(6), der(7), der(17), der(21), dic(13,14), and dic(14,18). Amplification of the C-MYC oncogene at 8q24 in two marker chromosomes: dup(8)(q24) and der(15)t(8,15)(q22,p11).

Handling

Culture Medium	DMEM
Medium supplements	10% FBS, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO3, w: 1.0 mM Sodium pyruvate
Passaging solution	Accutase
Subculturing	Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10 ml for T75 cell culture flasks). Add Accutase (1-2 ml per T25, 2.5 ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at ambient temperature for 8-10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3 min at 300 g, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
Split ratio	A ratio of 1:3 to 1:8 is recommended

Seeding density	1 x 10^4 cells/cm^2 will result in a confluent monolayer within 4 days.
Fluid renewal	2 to 3 times per week
Freezing recovery	After thawing, plate the cells at 5 x 10^4 cells/cm^2 and allow the cells to recover from the freezing process and to adhere for at least 24 hours.
Freeze medium	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)
Handling of cryopreserved cultures	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300 x g for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Resuspend the cells carefully in 10 ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the subculture section.
Handling of proliferating cultures	One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Incubate at 37 degree Celsius for a minimum of 24 hours.

Quality control / Genetic profile / HLA

Sterility	Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.

STR profile	**Amelogenin**: x,x **CSF1PO**: 11,12 **D13S317**: 9,13 **D16S539**: 12,14 **D5S818**: 12,13 **D7S820**: 10 **TH01**: 9 **TPOX**: 11 **vWA**: 15,17 **D3S1358**: 14 **D21S11**: 28,3
HLA alleles	**A***: 03:01:01 **B***: 07:02:01 **C***: 07:02:01 **DRB1***: 11:04:01 **DQA1***: 05:05:01 **DQB1***: 03:01:01 **DPB1***: 15:01:01 **E**: 01:03:01, 01:03:02