Product sheet

NCI-H295R growing culture | 330483

General information

Description	H295R was adapted from the NCI-H295 pluripotent adrenocortical carcinoma cell line established by A.F. Gazdar and associates (1990) from a carcinoma of the adrenal cortex. The original cells were adapted to a culture medium which decreased the population doubling time from 5 days to 2 days. The adapted cells were selected to grow in a monolayer, in contrast to the original cells which grew in suspension. This cell line retains the ability to produce adrenal androgens. It is responsive to angiotensin II and potassium ions.
Organism	Human
Tissue	Adrenal gland
Disease	Carcinoma
Synonyms	NCI-H295R, NCI H295R, NCIH295R, H-295R, H295R-S1

Characteristics

Age	48 years
Gender	Female
Ethnicity	Caucasian
Morphology	Epithelial-like
Growth properties	Monolayer, adherent

Identifiers / Biosafety / Citation

Citation	H295R (Cytion catalog number 300483)
Biosafety level	1

Expression / Mutation

Products	Aldosterone, cortisol, C19 steroids

Handling

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Culture Medium	DMEM:Ham's F12
Medium supplements	2% Nu-Seru, 10% FBS, w: 3.1 g/L Glucose, w: 1.6 mM L-Glutamine, w: 15 mM HEPES, w: 1.0 mM Sodium pyruvate, w: 1.2 g/L NaHCO3, 0.00625 mg/ml insulin, 0.00625 mg/ml transferrin, 6.25 ng/ml selenium, 1.25 mg/ml bovine serum albumin, 0.00535 mg/ml linoleic acid
Passaging solution	Accutase
Subculturing	Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks). Add Accutase (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at ambient temperature for 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 1:4 is recommended
Fluid renewal	2 to 3 times per week
Freezing recovery	48 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**: 10,12 **D13S317**: 13 **D16S539**: 11 **D5S818**: 12 **D7S820**: 9,12 **TH01**: 9.3 **TPOX**: 8 **vWA**: 17,18 **D3S1358**: 15,16 **D21S11**: 32.2 **D18S51**: 17 **Penta E**: 5,12 **Penta D**: 8 **D8S1179**: 13 **FGA**: 19.2,24
HLA alleles	**A***: 02:01:01 **B***: 15:10:01 **C***: 03:04:02 **DRB1***: 01:01:01 **DQA1***: 01:01:01 **DQB1***: 05:01:01 **DPB1***: 04:02:01 **E**: 01:03:02