

**Designation: 2427T**

CLS order number: Cryovial: 300167
Vital: 330167

Origin and General Characteristics	
Depositor:	M. Meister, Thoraxklinik Heidelberg
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	64 years of age
Gender:	Female
Tissue:	Lung primary tumor
Morphology:	Small round, show a high nucleus-to-cytoplasm ratio and grow in clusters
Celltype:	Squamous Cell Carcinoma
Growth Properties:	Monolayer, adherent
Description:	The 2427T cell line has been established from pulmonary squamous cell carcinoma (SCC) of a patient by Dr. Sandra Gottschling and Dr. Michael Meister in 2009.
Culture Conditions and Handling	
Culture Medium:	DMEM:Ham's F12 (1:1) supplemented with 2 mM L-glutamine and 10% fetal bovine serum (MG-40, CLS order number 820400)
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 (about 20-26°C) for 10 minutes. Carefully resuspend the cells with medium (10 ml), resuspend the cells in fresh medium and dispense into new flasks which contain fresh medium.
Split ratio:	A ratio of 1 to 5 is recommended.
Fluid Renewal:	2 times weekly
Freeze Medium:	90% FCS/10% DMSO
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative; CNA- and TSA-Agar culture for 48 hrs: negative
Biosafety Level:	1
Special Features of the Cell line	
Tumorigenic:	Rapid tumor formation in nude mice reaching a tumor diameter of 1.5cm after 28 days.
Karyotype:	M-FISH profiles show a near triploid complex Karyotype
Cell Marker:	CD9 (+), CD34 (-), CD44 (-), CD45 (-), CD54 (-), CD56 (-), CD117 (-), SYP (-/+), NSE (-), CHGA (-), CK5/6 (-/+), CK7 (-).
DNA Profile (STR):	Amelogenin: X,X CSF1PO: 9 D13S317: 8 D16S539: 12 D5S818: 12 D7S820: 8,11 THO1: 7,9 TPOX: 10,11 vWA: 14,18
Isoenzymes:	CDA not expressed
Products:	Zytokeratin 5/6
References: S. Gottschling et al. Establishment and comparative characterization of novel squamous cell non-small cell lung cancer cell lines and their corresponding tumor tissue. Lung cancer 75: 45-57, 2012.	
Order requirement: Ahead of dispatch of the cell line, CLS GmbH must have received the MTA completed and signed.	

Recommendations for handling of adherent cell cultures following delivery

Cryopreserved cells

If immediate culturing is not intended, the cryovial(s) must be stored in liquid nitrogen (-196°C) or at least at -80°C after arrival.

If immediate culturing is intended, please follow these instructions:

Quickly thaw by rapid agitation in a 37°C 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. Note: 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 300xg for 3-5 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 10ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the Subculture section.

Proliferating Cultures

The cell culture flasks are completely filled with cell culture medium to prevent loss of cells during transit.

Remove the entire medium except for a sufficient volume to cover the floor of the flask. Incubate at 37°C for 24 hrs.

Sometimes the cultures are handled roughly during transit, and most of the cells detach and float in the culture medium. If this has occurred remove the entire content of the flask and centrifuge at 300x g for 5 minutes. Take off the supernatant, resuspend the cells in 10 ml of culture medium and transfer the entire cell suspension into cell culture flasks of suitable size (do not seed in more than 1 T75 flask).

Safety precautions for frozen cell lines

If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed:

- Protective gloves and clothing should be used and a facemask or safety goggles must be worn when storing and/or thawing the cryovial.
- The removal of a cryovial from liquid nitrogen can result in the explosion of the cryovial creating flying fragments.

References: Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.