Designation: **HROG05**

CLS order number: Cryovial: 300940
Vital: 330940

Origin and General Characteristics		
Depositor:	Michael Linnebacher	
Organism:	Homo sapiens (human)	
Ethnicity:	Caucasian	
Age:	60 years	
Gender:	Female	
Tissue:	Brain, L; temporal	
Morphology:	Epithelial, fibroblast-like	
Cell type:	Relapsed Glioblastoma (grade IV)	
Growth Properties:	Adherent 2D, in colonies	
Description:	This is one cell line of a series of tumor cell lines which have been established by PD Dr. Michael Linnebacher from Primary CRC resection specimens since 2006.	
Culture Conditions and Handling		
Culture Medium:	DMEM/Ham's F12 with L-glutamine medium supplemented with 3 mM L-glutamine and 10% fetal bovine serum.	
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 TrypLE Express (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at 37°C for 10 to 15 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium. This cell line will result in single cell suspension.	
Split Ratio:	A ratio of 1:3 to 1:6 is recommended	
Seeding density:	$1x10^4/cm^2$	
Fluid Renewal:	1 to 2 times weekly	
Doubling time:	44 h / 48 h	
Freeze Medium:	CM-ACF (CLS order number 800650, 50ml)	
Sterility:	Mycoplasma specific PCR: negative; Mycoplasma specific PlasmoTest: negative; Bacteria, fungi: negative	
Biosafety Level:	1	
Special Features of the Cell Line		
DNA Profile (STR):	Amelogenin: X,X D3S1358: 15,16 D1S1656: 14,15.3 CSF1PO: 11,12 D13S317: 11,12 D16S539: 11,12 D5S818: 10,11 D21S11: 30,31.2 D2S1338: 17,25 D18S51: 14,16	D7S820: 7,11 D6S1043: 12,19 THO1: 6,7 TPOX: 8,11 vWA: 18,19 Penta D: 8,13 Penta E: 12,16 D8S1179: 13 D12S391: 23 D19S433: 13,14 FGA: 20,22
Tumor antigen	EGFR$^+$, HER2/neu $^-$, Survivin $^+$, MAGE-1 $^-$, MART-1 ^{low}, Tyrosinase $^-$, RHAMM^{low}, WT-1 $^+$, IL-13Ra $^-$	

Cell Marker:	HLA-A02^{+}, MHC class I -IFN-γ $^{+}$ IFN-γ $^{+}$, MHC class II $^{-}$ IFN-γ $^{+}$ IFN-γ $^{+}$, ICAM-1 $^{+}$ ß-microglobulin $^{+}$, Beta-2-M^{+}, HLA-E^{+}, HLA-G ^{low}, , MIC A ^{low}, MIC-B $^{-}$, GFAP^{+} ; nestin $^{+}$, vimentin +, S-100^{+}, GBM^{+}, BTSC^{+}
Mutations :	IDH 1 & 2 ^{wt}, TP53^{wt}, K-Ras ^{G12D}, B-RAF^{wt} , 4q12(PDGFRA) amplified, PTEN $^{P169S/del\ 212-229}$
MGMT promotor :	Methylated
ASS1 :	Methylated
ASL :	Low methylated, low unmethylated
Arginine Diminase (ADI) response :	Positive
Cytokine secretion:	IL-8^{high}, IL-6^{high}, CEA^{-}, TNF alpha $^{-}$, TGF beta^{-}

References:

Mullins CS, Schneider B, Stockhammer F, Krohn M, Classen CF and Linnebacher M. (2013) Establishment and Characterization of Primary Glioblastoma Cell Lines from Fresh and Frozen Material: A Detailed Comparison. PLoS ONE 8(8): e71070. Doi:10.1371/journal.pone.0071070.

Mullins C.S., Walter A., Schmitt M., Classen C.-F., Linnebacher M., Tumor antigen and MHC expression in glioma cells for immunotherapeutic interventions, World Journal fo Immunology, November 27, 2013, Vol. 3, Issue 3

Mullins C.S., Schneider B., Lehmann A., Stockhammer F., Mann S., Classen C.-F.and Linnebacher M., J. Cancer Sciu Ther. 2014, Vol6/6) 177-187

Fiedler T., Strauss M., Hering S., Redanz U., Wiilliom D., Rosche Y., Classen C.-F., Kreikemeyer B., Linnebacher M. and Maletzki C., Arginine deprivation by argininge deiminase of Streptococcus pyogenes controls primary glioblastoma growth in vitro an din in vivo; Cancer Biology & Therapy 16:7, 714-722; July 2015 Tylor & Francis Group, LLC

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 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 1T75 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.