Designation: **Kelly**

CLS order number: Cryovial: 300317
Vital: 330317

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Tissue:	Brain
Morphology:	Adherent
Cell type:	Neuroblastoma
Growth Properties:	Monolayer, adherent
Description:	The Kelly cell line has been established from a patient with neuroblastoma. They possess a genomic amplification of the N-myc gene.
Culture Conditions and Handling	
Culture Medium:	RPMI 1640 medium supplemented with 2mM L-glutamine and 10% fetal bovine serum (MG-70, CLS order number 820700).
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 5 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
Split Ratio:	A ratio of 1:6 to 1:8 is recommended
Seeding density:	$1x10^4$ cells/cm^2
Fluid Renewal:	2 to 3 times weekly
Doubling time:	Approx. 30 hours
Freeze Medium:	CM-2 (CLS order number 800250, 50ml)
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
Biosafety Level:	1
Special Features of the Cell Line	
Tumorigenic:	Yes, in nude mice
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR Negative for HPV (Human Papilloma Virus)
HeLa Markers:	None
DNA Profile (STR):	Amelogenin: X,X CSF1PO: 12 D13S317: 14 D16S539: 12,13 D5S818: 11,13 D7S820: 9 THO1: 9.3 TPOX: 8,10 vWA: 17,18 D3S1358: 14,16 D21S11: 28,30 D18S51: 14,17 Penta E: 12,16 Penta D: 9,14 D8S1179: 14 FGA: 20,21 D1S1656:11,13 D6S1043:12,13 D19S433:12,15.2 D12S391:19,19.3
Products:	N-myc RNA

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References:
Schwab M, Alitalo K, KLempnauer K-H, Varmus HE, Bishop JM, Gilbert F, Brodeur G, Goldstein M, Trent J. Amplified DNA with limited homology to myc cellular oncogine is shared by human neuroblastoma cell lines and a neuroblastome tumour. Nature 305: 245 - 248, 1983.

Recommendations for handling of adherent cell cultures following delivery

Cryopreserved cells

If immediate culturing is not intended, the cryovial(s) may be stored in liquid nitrogen 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 (a small ice clump should remain and the cryovial 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. Resuspend the cells carefully. Centrifuge at 300xg for 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 fresh cell culture medium and transfer them into two T25 cell culture flasks. To ensure rapid recovery it is recommended to seed the cells at between 1/4 -1/2 of their maximum density. In practice the maximum density of many suspension lines is 10^6 /ml and for adherent lines 1-3 x 10^5 cells/cm^2. Incubate at 37°C / 5% CO_2.

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 3 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.