Designation: **Y-1**

CLS order number: Cryovial: 400477
Vital: 440477

Origin and General Characteristics	
Depositor:	CLS
Organism:	Mus musculus (mouse)
Strain:	LAF1
Gender:	Male
Tissue:	Adrenal gland
Morphology:	Epithelial
Cell type:	Adrenocortical tumor
Growth Properties:	Adherent
Description:	The Y-1 cell line was originally cloned by Yasumura et al. (1966) from mouse adrenal cortex tumor cultures which had been established by Buonnassisi et al. (1962).
Culture Conditions and Handling	
Culture Medium:	DMEM: Ham's F12 medium (1:1 mixture) supplemented with 2mM L-glutamine and 5% 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 for 8-10 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.
Split Ratio:	1:2 to 1:6 is recommended
Fluid Renewal:	at least 2 times per week (or else hormone production will decrease)
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
Biosafety Level:	1
Special Features of the Cell Line	
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR
Authentication :	The mouse origin was verified by Real-Time PCR.
Karyotype :	modal number = 41; range = 40 to 108. Stemline number is hyperdiploid. Karyotype stable within stemline number. Thirty-nine chromosomes with terminal centromeres plus 2 minute chromosomes; some cells with only l minute chromosome. The first chromosome is quite large and some of the smaller chromosomes are smaller than normal, but no specific structural abnormalities were detected.
Products :	steroid hormones

References:

Yasumura Y et al. Clonal analysis of differentiated function in animal cell cultures. I. Possible correlated maintenance of differentiated function and the diploid karyotype. Cancer Res 26: 529-35, 1966.

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 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.