Designation: **HBL-100**

CLS order number: Cryovial: 300178
Vital: 330178

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	caucasian
Age:	27 years of age
Gender:	Female (see NOTE under Karyotype)
Tissue:	Breast
Morphology:	Epithelial
Cell type:	Mammary gland
Growth Properties:	Monolayer, adherent
Description:	The epithelial cell line HBL-100 has been derived by E.V. Gaffney and associates from the milk of a nursing mother and obtained 3 days after delivery. Although there was no evidence of a breast lesion in the milk donor, and the patient had no family history of breast cancer, the karyotype of the recovered cells was abnormal as early as passage 7. This line was able to synthesize a small amount of lactose and would respond to prolactin or estrogen by producing increased amounts of casein. Electron micrographs revealed microvilli, tonofibrils and desmosomes.
Culture Conditions and Handling	
Culture Medium:	DMEM supplemented with 4.5g/L glucose, L-glutamine, and 10% fetal bovine serum (MG-30, CLS order number 820300).
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:	A ratio of 1:2 is recommended
Fluid Renewal:	2 to 3 times weekly
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:	2
Special Features of the Cell Line	
Tumorigenic:	Yes, in nude mice. At passage levels below 35 the line is not tumorigenic in nude mice, but forms colonies in soft agar. Tumorigenicity has been reported to increase above passage 35.
Viruses:	The cells contain a tamdemly integrated SV40 genome; it has been reported that they may contain a type D retrovirus that is similar or identical to Mason-Pfizer monkey virus (MPMV). SMRV: Negative, as confirmed by Real-Time PCR
Karyotype:	The stemline chromosome number is near triploid with the modal number of 67 chromosomes, and the 2S component occurring at 0.6%. Most chromosome complements consist of about 39 normal and 28 marker chromosomes. Markers such as 2q, 11q+, 11q, t(2q;12), t(2q;5q?), t(6p?;16), 16pt and many others are common to most metaphases. Normal chromosomes 11, 14, 15 and 16 are absent; 2, 12, 17 and 19 are monosomic, and the X is disomic. **NOTE:** According to reports by ATCC, DNA profiling for amelogenin, a sex-chromosome-specific PCR assay that can distinguish X chromosome-specific products from Y

	chromosome-specific products revealed the presence of Y chromosomes in this cell line of putative female origin. Confirmation of the general findings was accomplished by QM staining, C-banding, and FISH, with a whole chromosome paint probe to the human Y chromosome.	
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10 D13S317: 12 D16S539: 9,12 D5S818: 11,12 D7S820: 8,12 THO1: 6,8 TPOX: 8	vWA: 16 D3S1358: 14,16 D21S11: 28,30 D18S51: 16 Penta E: 7 Penta D: 12 D8S1179: 12,15 FGA: 25
Antigen Expression:	HLA A1, A10, A11, B7, B8	
Isoenzymes:	G6PD, B; PGM1, 1; PGM3, 2; ES-D, 1; Me-2, 0; GLO-1, 2; AK-1, 1-2; Phenotype Frequency Product: 0.0008	
Reverse Transcriptase:	positive	

References:
Gaffney EV, Blackburn SE, Polanowski FP. The hormone response of secreting and nonsecreting humen breast cells in culture. In Vitro 12: 328-329, 1976.

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.