Product sheet

HBL-100 | 300178

General information

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. Problematic cell line: Misidentified. Presence of a Y chromosome in cell line that was thought to be of female origin (Yoshino et al. 2006. Capes-Davies, 2010). Originally thought to originate from a casein-producing breast cell line. In addition contains SV40 genomic sequence while the cell line was deemed to be spontaneously immortalized.
Organism	Human
Tissue	Breast
Disease	Carcinoma
Synonyms	HBL 100, HBL100

Characteristics

Age	27 years
Gender	Female
Ethnicity	Caucasian
Morphology	Epithelial-like
Growth properties	Monolayer, adherent

Identifiers / Biosafety / Citation

Citation	HBL-100 (Cytion catalog number 300178)
Biosafety level	1

Expression / Mutation

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
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).
Reverse transcriptase	Positive
Ploidy status	Aneuploid
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. 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.

Handling

Culture Medium	McCoys 5a
Medium supplements	10% FBS, w: 3.0 g/L Glucose, w: stable Glutamine, w: 2.0 mM Sodium pyruvate, w: 2.2 g/L NaHCO3
Passaging solution	Accutase
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

Seeding density	1 x 10^4 cells/cm^2
Fluid renewal	2 to 3 times per week
Freezing recovery	After thawing, plate the cells at 5 x 10^4 cells/cm^2 and allow the cells to recover from the freezing process and to adhere for at least 24 hours.
Freeze medium	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)
Handling of cryopreserved cultures	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius 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 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 300 x g 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 10 ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the subculture section.
Handling of proliferating cultures	One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Incubate at 37 degree Celsius for a minimum of 24 hours.

Quality control / Genetic profile / HLA

Sterility	Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.

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STR profile	**Amelogenin**: x,Y **CSF1PO**: 10 **D13S317**: 12 **D16S539**: 9,12 **D5S818**: 11,12 **D7S820**: 8,12 **TH01**: 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
HLA alleles	**A***: 01:01:01, 02:01:01 **B***: 08:01:01, 40:01:02 **C***: 03:04:01, 07:01:01 **DRB1***: 03:01:01, 15:01:01 **DQA1***: 01:02:01, 05:01:01 **DQB1***: 02:01:01, 06:02:01 **DPB1***: 04:01:01 **E**: 01:01, 01:03