

#### **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
lsoenzymes	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, w: 3.0 g/L Glucose, w: stable Glutamine, w: 2.0 mM Sodium pyruvate, w: 2.2 g/L NaHCO3 (Cytion article number 820200a)
Medium supplements	Supplement the medium with 10% FBS
Passaging solution	Accutase
Subculturing	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already 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	1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
	2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
	3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
	4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
	5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
	6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
	7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
	8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

## Quality control / Genetic profile / HLA



Sterility	Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods. To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.
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