**Designation:** **HBL-100**

CLS order number: Cryovial: 300178
Vital: 330178

| Origin and General Characteristics | |
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| 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.<br>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. |
| 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.<br>Yoshino K., Iimura E., Saijo K., Iwase S., Fukami K., Ohno T., Obata Y., Nakamura Y. Essential role for gene profiling analysis in the authentication of human cell lines. Hum. Cell 19:43-48, 2006.<br>Capes-Davis A., Theodosopoulos G., Atkin I., Drexler H.G., Kohara A., MacLeod R.A.F., Masters J.R.W., Nakamura Y., Reid Y.A., Reddel R.R., Freshney R.I. Check your cultures! A list of cross-contaminated or misidentified cell lines. Int. J. Cancer 127:1-8, 2010. |
| 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).<br>Add Accutase (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely.<br>Incubate at ambient temperature for 8-10 minutes.<br>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: | $1 \times 10^4$ cells/$cm^2$ |
| Fluid Renewal: | 2 to 3 times weekly |
| Doubling time: | About 40 hrs |
| Freeze Medium: | CM-1 (CLS order number: 800125, 25ml, 800150, 50ml) |

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| Freezing recovery: | Following thawing, seed the cells at 4-5 x $10^4$ cells/$cm^2$ and allow to recover from the freezing process for at least 24 hrs. |
| Sterility: | Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative |
| Biosafety Level: | 1 |
| Safety precautions: | If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed:<br>Protective gloves and clothing should be used and a facemask or safety goggles must be worn when transferring frozen samples into or removing from the liquid nitrogen tank.<br>The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments.<br>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. |
| 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.<br>**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<br>CSF1PO: 10<br>D13S317: 12<br>D16S539: 9,12<br>D5S818: 11,12<br>D7S820: 8,12<br>THO1: 6,8<br>TPOX: 8<br>vWA: 16<br>D3S1358: 14,16<br>D21S11: 28,30<br>D18S51: 16<br>Penta E: 7<br>Penta D: 12<br>D8S1179: 12,15<br>FGA: 25 |
| Ploidy status: | Aneuploid |
| MSI-status: | Stable (MSS) |
| 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 |

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| Certificate of Analysis: | The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de. |

Recommendations for handling of adherent cell cultures following delivery

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| Cryopreserved cells | The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen.<br>If immediate culturing is not intended, the cryovial(s) must be stored below -150°C after arrival.<br>If immediate culturing is intended, please follow these instructions:<br>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.<br>From now on, all operations should be carried out under aseptic conditions.<br>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.<br>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, 2xT25, come filled with cell culture medium.<br>Collect the entire medium in 2x 50 ml centrifuge tubes.<br>Carefully add 5 ml of cell culture medium to each of the two T25 cell culture flasks.<br>Control the cell morphology and confluency under the microscope.<br>Incubate at 37°C for a minimum of 24 hrs.<br>Spin down the collected medium at 300x 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 1xT25 cell culture.<br>Incubate at 37°C for a minimum of 24 hrs. |

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| Warranty: | CLS warrants for a high cell viability and culture performance only if the product(s) is (are) stored and cultured according to the information described above. Using cell culture media and supplements other than the ones recommended in this product information may result in satisfactory proliferation and viabilities. CLS, however, does not warrant for cell recovery, proliferation and function if differing formulations are employed. |
| Disclaimer: | The customer shall not be entitled to employ this product for purposes other than research. Commercial utilization shall not be permitted; in particular, the cell line, its components or materials made therefrom shall not be sold or transferred to any third party. In addition, the term ‘Commercial use’ shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components in manufacturing, for providing services, e.g. fee for service testing, in quality control or assurance processes within the manufacturing of products for sale, for therapeutic, diagnostic or prophylactic purposes, or for resale. |