**Designation:** **B-LCL-CDG4**

CLS order number: Cryovial: 302015
DNA: 302015GD

| Origin and General Characteristics | |
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| Organism: | Homo sapiens |
| Ethnicity: | Caucasian |
| Gender / Age | Female / Child |
| Tissue: | Hematopoietic system |
| Disease: | CDAII (Congenital Disorder of Glycosylation or Congenital Dyserythropoetic Anemia type II, formerly designated HEMPAS=hereditary erythroblastic multinuclearity with positive acidified serum lysis test) |
| Morphology: | B-Lymphoblast |
| Growth Properties: | Suspension, Cluster |
| Description: | B-LCL-CDG4 is an EBV-transformed B lymphocyte cell line derived from a young girl with CDAII. CDAII is a rare genetic anaemia, affiliated to the class of CDG glycosylation disorders. CDAII patients have a defect in the COPII component SEC23B gene which is involved in the intracellular protein transport system (in particular vesicular budding from ER). The respective patient is homozygous for the mutation in this gene. Band 3 glycoprotein of erythrocyte membranes is under glycosylated by aberrant glycosylation of polylactosamine motifs of glycoproteins but not of glycosphingolipids, thus band 3 of CDA II erythrocytes have truncated hybrid-type oligosaccharides. This points to an additional defect in the Golgi glycosylation enzymes $\alpha$-mannosidase II or N-acetylglucosaminyltransferase II. |
| References: | Van Schaftingen E, Jaeken J. Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I. FEBS Lett 377: 318-320 (1995).<br>Bergmann M, Gross HJ, Abdelatty F, Möller P, Jaeken J, Schwartz-Albiez R. Abnormal surface expression of sialoglycans on B lymphocyte cell lines from patients with carbohydrate deficient glycoprotein syndrome I A (CDGS I A). Glycobiology. 8(10):963-72 (1998).<br>Denecke J, Kranz C, Nimtz M, Conradt HS, Brune T, Heimpel H, Marquardt T. Characterization of the N-glycosylation phenotype of erythrocyte membrane proteins in congenital dyserythropoietic anemia type II (CDA II/HEMPAS). Glycoconj J. 25(4):375-382. doi: 10.1007/s10719-007-9089-1 (2008).<br>Jaeken J, Hennet T, Matthijs G, Freeze HH. CDG nomenclature: time for a change! Biochim Biophys Acta 1792(9): 825-826 (2009).<br>Bianchi P, Fermo E, Vercellati C, Boschetti C, Barcellini W, Iurio A, Marcello AP, Righetti PG, Zanella A. Congenital dyserythropoietic anemia II (CDAII) is caused by mutations in the SEC23B gene. Hum Mutat 30(9): 1292-1298 (2009).<br>Thiel C, Körner C. Therapies and therapeutic approaches in Congenital Disorders of Glycosylation. Glycoconj J.;30(1):77-84. doi: 10.1007/s10719-012-9447-5. (2012).<br>Monticelli M, Ferro T, Jaeken J, Dos Reis Ferreira V, Videira PA. Immunological aspects of congenital disorders of glycosylation (CDG): a review. J Inherit Metab Dis 39(6):765-780 (2016).<br>Péanne R, de Lonlay P, Foulquier F, Kornak U, Lefeber DJ, Morava E, Pérez B, Seta N, Thiel C, Van Schaftingen E, Matthijs G, Jaeken J. Congenital disorders of glycosylation (CDG): Quo vadis? Eur J Med Genet. 61(11):643-663. doi: 10.1016/j.ejmg.2017.10.012. (2017).<br>Jaeken L. The neglected functions of intrinsically disordered proteins and the origin of life. Prog Biophys Mol Biol. 126:31-46. doi: 10.1016/j.pbiomolbio.2017.03.002. (2017).<br>Ferreira CR, Altassan R, Marques-Da-Silva D, Francisco R, Jaeken J, Morava E. Recognizable phenotypes in CDG. J Inherit Metab Dis. 41(3):541-553. doi: 10.1007/s10545-018-0156-5. (2018).<br>Ng BG, Freeze HH. Perspectives on Glycosylation and Its Congenital Disorders. Trends Genet 34(6):466-476. doi: 10.1016/j.tig.2018.03.002. Epub (2018). |

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| | Joshi HJ, Hansen L, Narimatsu Y, Freeze HH, Henrissat B, Bennett E, Wandall HH, Clausen H, Schjoldager KT. Glycosyltransferase genes that cause monogenic congenital disorders of glycosylation are distinct from glycosyltransferase genes associated with complex diseases. Glycobiology 28(5):284-294 (2018).<br>Pascoal C, Francisco R, Ferro T, Dos Reis Ferreira V, Jaeken J, Videira PA. CDG and immune response: From bedside to bench and back. J Inherit Metab Dis.. doi: 10.1002/jimd.12126 (2019).<br>Fukuda MN. HEMPAS. Hereditary erythroblastic multinuclearity with positive acidified serum lysis test. Biochim Biophys Acta 1455(2-3): 231-239 (1999). |
| Culture Conditions and Handling | |
| Culture Medium: | RPMI 1640 medium, supplemented with 10% FBS (CLS order number 820700a, basic medium, or 820700 ready-to-use). |
| Subculturing: | Maintain culture between 3 to 5 x$10^5$ cells/ml. Incubate at 5% $CO_2$, 37°C. |
| Seeding density: | n.a. |
| Fluid Renewal: | 1-2 times weekly |
| Freeze Medium: | CM-1 (CLS order number: 800125, 25ml, 800150, 50ml) |
| Freezing recovery: | Medium to Fast |
| Sterility: | Mycoplasma specific qPCR and cell-based assay: negative<br>Bacteria control: negative |
| Biosafety Level: | 2<br>B-LCL-CDG4 was tested positive for EBV. According to the German Law for the Protection against Infections (Infektionsschutzgesetz IfSG), this cell line falls under Risk group L2, and can only be distributed to customers holding a valid permit of the respective authority (IfSG §44 and 45). |
| 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 | |
| Surface antigens: | CD19+, CD20+, CD37+, CD43+, CD44+, CD45+, CD45R0-;<br>MHC Cl.I+, MHC Class II (HLA-DR)+ |
| Carbohydrate antigens | CD15 (Lewis x)+, CD15s (sialylated Lewis x)-, CD75s (sialylated lactosaminyl N-oligosccharides)+, CD173 (blood group H)-, CD174 (blood group Lewis y)-, CD175 (Tn)-, CD175s (sialylated Tn)-, CD176 (TF)+ |
| DNA Profile (STR) : | Amelogenin: X,X<br>CSF1PO: 11,12<br>D13S317: 8,13<br>D16S539: 11,12<br>D5S818: 11,11<br>D7S820: 8,14<br>TH01: 6,9<br>TPOX: 8,8<br>D3S1358: 16,17<br>D21S11: 30,30<br>D18S51: 14,16<br>Penta E: 7,19<br>Penta D: 8,12<br>D8S1179: 13,13<br>FGA: 23,23.2<br>vWA: 16,16 |

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| HLA-typing: | Class Ia<br>A*01:01:01, A*24:02:01<br>B*08:01:01, B*18:01:01<br>C*07:01:01, C*12:03:01<br>Class Ib<br>E*01:01, *01:03 | Class II<br>DRB1*03:01:01, *15:01:01<br>DQA1*01:02:01, *05:01:01<br>DQB1*02:01:01, *06:02:01<br>DPB1*03:01:01, *04:02:01 |
| Applications: | Genotyping of CDG effects in immune cells; functional testing (e.g. B cell surface antigens); testing of cytotoxic drugs; mutational analysis; analysis of apoptotic mechanisms; HLA-typing; impact of defective glycosylation of distinct cellular glycoproteins on diverse functions. | |

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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 cells growing in suspension 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 one T25 cell culture flask. All further steps are described in the Subculture section. |
| Proliferating Cultures | This cell line is not available as vital culture. |

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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. |