

**Designation: B-LCL-CDG2**

CLS order number: Cryovial: 302013
DNA: 302013GD

Origin and General Characteristics	
Organism:	Homo sapiens
Ethnicity:	Caucasian
Gender / Age:	Female / Child
Tissue:	Hematopoietic system
Disease:	CDG (Congenital Disorders of Glycosylation) Type PMM2-CDG (formerly known as CD1a)
Morphology:	B-Lymphoblast
Growth Properties:	Suspension, Cluster
Description:	B-LCL-CDG2 is an EBV-transformed B lymphocyte cell line derived from a young girl suffering from PMM2-CDG. PMM2-DG is a rare inborn error of metabolism, which results in defective synthesis of glycosylated oligosaccharide chains of many tissue and blood glycoproteins and/or glycosphingolipids. The primary cause of defective glycosylation is based on mutations in the enzyme phosphomannomutase 2 (PMM2). The respective patient is homozygous for the mutation in PMM2.
References:	<p>Van Schaftingen E, Jaeken J. Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I. <i>FEBS Lett</i> 377: 318-320 (1995).</p> <p>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). <i>Glycobiology</i>. 8(10):963-72 (1998).</p> <p>Jaeken J, Hennot T, Matthijs G, Freeze HH. CDG nomenclature: time for a change! <i>Biochim Biophys Acta</i> 1792(9): 825-826 (2009).</p> <p>Schneider A, Thiel C, Rindermann J, DeRossi C, Popovici D, Hoffmann GF, Gröne HJ, Körner C. Successful prenatal mannose treatment for congenital disorder of glycosylation-Ia in mice. <i>Nat Med</i> 18(1):71-3. doi: 10.1038/nm.2548 (2011).</p> <p>Thiel C, Körner C. Therapies and therapeutic approaches in Congenital Disorders of Glycosylation. <i>Glycoconj J.</i>;30(1):77-84. doi: 10.1007/s10719-012-9447-5. (2012).</p> <p>Rymen D, Winter J, Van Hasselt PM, Jaeken J, Kasapkara C, Gokçay G, Haijes H, Goyens P, Tokatli A, Thiel C, Bartsch O, Hecht J, Krawitz P, Prinsen HC, Mildenerberger E, Matthijs G, Kornak U. Key features and clinical variability of COG6-CDG. <i>Mol Genet Metab</i>. 116(3):163-70. doi: 10.1016/j.ymgme.2015.07.003. (2015).</p> <p>Monticelli M, Ferro T, Jaeken J, Dos Reis Ferreira V, Videira PA. Immunological aspects of congenital disorders of glycosylation (CDG): a review. <i>J Inherit Metab Dis</i> 39(6):765-780 (2016).</p> <p>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? <i>Eur J Med Genet</i>. 61(11):643-663. doi: 10.1016/j.ejmg.2017.10.012. (2017).</p> <p>Jaeken L. The neglected functions of intrinsically disordered proteins and the origin of life. <i>Prog Biophys Mol Biol</i>. 126:31-46. doi: 10.1016/j.pbiomolbio.2017.03.002. (2017).</p> <p>Ferreira CR, Altassan R, Marques-Da-Silva D, Francisco R, Jaeken J, Morava E. Recognizable phenotypes in CDG. <i>J Inherit Metab Dis</i>. 41(3):541-553. doi: 10.1007/s10545-018-0156-5. (2018).</p> <p>Ng BG, Freeze HH. Perspectives on Glycosylation and Its Congenital Disorders. <i>Trends Genet</i> 34(6):466-476. doi: 10.1016/j.tig.2018.03.002. Epub (2018).</p> <p>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. <i>Glycobiology</i> 28(5):284-294 (2018).</p>

	<p>Altassan R, Péanne R, Jaeken J et al. International clinical guidelines for the management of phosphomannomutase 2-congenital disorders of glycosylation: Diagnosis, treatment and follow up. J Inherit Metab Dis. 42(1):5-28. doi: 10.1002/jimd.12024 (2019).</p> <p>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).</p>	
Culture Conditions and Handling		
Culture Medium:	RPMI 1640 supplemented with 10% FBS (CLS order number 820700a basic medium, 820700 ready-to-use).	
Subculturing:	Maintain culture between 2 to 5 x10 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 Bacteria control: negative	
Biosafety Level:	2 B-LCL-CDG2 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: 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. The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments. 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:	CD10-, CD19+, CD20+, CD21+, CD22+, CD23+, CD24+, CD37+m CD38+, CD39+, CD40+, CD53+, CD71+, CD72(+), CD73+, CD74 (+), CD80+, CD81+, CD82+, CD83-, CD84-, CD85+, CD86+; MHC class I+, MHC class II+	
Carbohydrate antigens:	CD60a- (GD3), CD60c-(7-O-acetylated GD3), CD75s+ sialylated lactosaminy! N-oligosaccharides), CD77- (Gb3, globotriaosylceramide)	
DNA Profile (STR):	Amelogenin: X,X CSF1PO: 9,12 D13S317: 11,14 D16S539: 13,14 D5S818: 11,12 D7S820: 9,10 TH01: 6,9.3 TPOX: 8,9	D3S1358: 17,17 D21S11: 29,31.2 D18S51: 16,17 Penta E: 7,10 Penta D: 9,12 D8S1179: 11,13 FGA: 22,24 vWA: 15,16
HLA-typing:	Class Ia A*02:01:01, A*31:01:02 B*40:01:02, B*44:02:01 C*03:04:01, C*05:01:01 Class Ib E*01:01, *01:03	Class II DRB1*04:04:01, *09:01:02 DQA1*03:01:01, *03:02:01 DQB1*03:02:01, *03:03:02 DPB1*04:02:01, *06:01:01

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 .
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Recommendations for handling of cells growing in suspension following delivery	
Cryopreserved cells	<p>The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen.</p> <p>If immediate culturing is not intended, the cryovial(s) must be stored below -150°C after arrival.</p> <p>If immediate culturing is intended, please follow these instructions:</p> <p>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.</p> <p>From now on, all operations should be carried out under aseptic conditions.</p> <p>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.</p> <p>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.</p>
Proliferating Cultures	This cell line is not available as vital culture.

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.