# B-LCL-CDG5

302016

## GENERAL INFORMATION

### Description

B-LCL-CDG5 is an EBV-transformed B lymphocyte cell line derived from a patient with PMM2-CDG, a congenital disorder of glycosylation (CDG) caused by mutations in the *PMM2* gene. This disorder impairs the proper synthesis and attachment of glycan structures to glycoproteins and glycolipids, affecting multiple organ systems. The deficiency in phosphomannomutase 2 (PMM2) disrupts the conversion of mannose-6-phosphate to mannose-1-phosphate, a critical step in glycosylation, leading to defects in cellular function and systemic complications.
As an EBV-immortalized B cell line, B-LCL-CDG5 serves as a crucial model for studying the biochemical and molecular effects of *PMM2* mutations. This cell line enables researchers to investigate glycosylation defects, PMM2 enzymatic activity, and the cellular consequences of impaired glycosylation. Additionally, it provides a platform for testing potential therapeutic approaches, such as pharmacological chaperones, enzyme enhancement therapies, or substrate supplementation strategies. B-LCL-CDG5, in combination with other CDG patient-derived cell lines, aids in advancing our understanding of PMM2-CDG and the development of targeted treatment options.

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| **Tissue** | Peripheral blood |
| **Disease** | EBV-transformed B lymphoblastoid cell line (B-LCL); PMM2-CDG (congenital disorder of glycosylation, patient-derived) |
| **Metastatic site** | Not applicable (EBV-transformed B-LCL; non-metastatic) |

### 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.

## CLASSIFICATION

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| **Organism** | Human |
| **Sex** | Female |
| **Growth properties** | Suspension |
| **GMO status** | GMO-S2 |

### GMO status details

GMO-S2: This B-LCL contains a stably maintained EBV episome encoding viral latent-phase genes (EBNA-1/-2/-3, LMP-1/-2). EBV is a risk group 2 pathogen; BSL-2 containment required. This classification applies within Germany; regulations may differ elsewhere.

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| **Biosafety level** | 2 |

## CHARACTERISTICS

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| **Morphology** | Round cells |
| **Cell type** | B lymphocyte |

## IDENTIFIERS

### Citation

B-LCL-CDG5 (Cytion catalog number 302016)

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| | |
|---|---|
| **NCBI TaxID** | 9606 |
| **Cellosaurus** | Not assigned |

## EXPRESSION AND SUSCEPTIBILITY

### Viruses

Transformant: EBV

## HANDLING AND CULTURE

### Culture medium

RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO3 (Cytion article number 820700a)

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| **Incubation atmosphere** | 37°C, 5% $CO_2$, humidified atmosphere. |

### Supplements

Supplement the medium with 10% heat-inactivated FBS

### Subculturing

Maintain cultures by periodically adding or replacing the medium. Initiate cultures with a density of 2 x $10^5$ cells/ml and keep the cell concentration within the range of 1 x $10^5$ to 5 x $10^5$ cells/ml for optimal growth.

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| **Fluid renewal** | Once the medium colour turned into yellow |
| **Post-thaw recovery** | Medium |

### Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
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.
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.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
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.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

## QUALITY CONTROL AND CRYOPRESERVATION

### 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.

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| **Freeze medium** | As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress. |

### Storage conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

### Shipping conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

## DONOR

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| **Ethnicity** | Caucasian |

## LEGAL AND SAFETY

**Safety precautions**

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

**Warranty**

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

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| **Material Transfer Agreement required** | Yes |

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