

**B-LCL-CDG4 Cells | 302015****General information**

**Description** B-LCL-CDG4 is an EBV-transformed B lymphocyte cell line derived from a young girl with CDAll. CDAll is a rare genetic anaemia, affiliated to the class of CDG glycosylation disorders. CDAll 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 poly lactosamine 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 mannosidase II or Nacetylglucosaminyltransferase II.

**Organism** Human

**Tissue** Peripheral blood

**Disease** Congenital Disorders of Glycosylation

**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.

**Characteristics**

**Age** Child

**Gender** Female

**Ethnicity** Caucasian

**Morphology** Round cells

**Cell type** B lymphocyte

**Growth properties** Suspension, Cluster

**Regulatory Data**

**Citation** B-LCL-CDG4 (Cytion catalog number 302015)

**Biosafety level** 2

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**NCBI\_TaxID** 9606

**CellosaurusAccession** CVCL\_A9Y2

### Biomolecular Data

**Surface antigens** CD15 (Lewis x)+, CD15s (sialylated Lewis x)-, CD75s (sialylated lactosaminyl Noligosaccharides)+, CD173 (blood group H)-, CD174 (blood group Lewis y)-, CD175 (Tn)-, CD175s (sialylated Tn)-, CD176 (TF)+

**Antigen expression** CD19+, CD20+, CD37+, CD43+, CD44+, CD45+, CD45R0-, MHC Cl.I+, MHC Class II (HLA-DR)+

**Viruses** Transformant: EBV

### Handling

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

**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  $2 \times 10^5$  cells/ml and keep the cell concentration within the range of  $3 \times 10^5$  to  $5 \times 10^5$  cells/ml for optimal growth.

**Fluid renewal** Once the medium colour turned into yellow

**Post-Thaw Recovery** Medium

**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.

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### Thawing and Culturing Cells

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  $-150^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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### **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.