

B-LCL-CDG7 Cells | 302018

General information

Description B-LCL-CDG7 is an EBV-transformed B lymphocyte cell line derived from a young boy 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 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 Beta-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 Male

Ethnicity Caucasian

Morphology Round cells

Cell type B lymphocyte

Growth properties Suspension, Cluster

Identifiers / Biosafety / Citation

Citation B-LCL-CDG7 (Cyton catalog number 302018)

Biosafety level 2

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Expression / Mutation

Surface antigens CD15 (Lewis x)(+), CD15s (sialylated Lewis x)-, CD75s (sialylated lactosaminyl N oligosaccharides)+, 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 Class.I+, MHC Class II (HLA-DR)+

Handling

Culture Medium RPMI 1640, w: 2.1 mM stable Glutamine, w: 2.0 g/L NaHCO₃ (Cytion article number 820700a)

Medium 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 2×10^5 cells/ml and keep the cell concentration within the range of 1×10^5 to 5×10^5 cells/ml for optimal growth.

Fluid renewal Once the medium colour turned into yellow

Freezing recovery Medium

Freeze medium CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

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Handling of cryopreserved cultures

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°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°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 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
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.

Quality control / Genetic profile / HLA

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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STR profile

Amelogenin: x,y
CSF1PO: 11
D13S317: 12, 14
D16S539: 10, 12
D5S818: 11, 12
D7S820: 8, 10
TH01: 6, 7
TPOX: 8, 11
vWA: 17, 18
D3S1358: 17, 18
D21S11: 30
D18S51: 13, 16
Penta E: 7, 12
Penta D: 9, 14
D8S1179: 11, 13
FGA: 21, 24

HLA alleles

A*: 01:01:01, 11:01:01
B*: 35:01:01, 51:01:01
C*: 01:02:01, 04:01:01
DRB1*: 07:01:01, 09:01:02G
DQA1*: 02:01:01, 03:02:01
DQB1*: 02:02:01, 03:03:02
DPB1*: 02:01:02G, 04:02:01G
E: 01:01:01