B-LCL-HROC69

300864

GENERAL INFORMATION

Description

B-LCL-HROC69 is an Epstein-Barr virus (EBV)-immortalized B lymphoblastoid cell line established from tumor-infiltrating B cells (TiBc) isolated from a primary colorectal carcinoma specimen designated HROC69. The parental tumor originated from an adult male patient with right-sided colorectal carcinoma of conventional sporadic type and advanced stage disease. B cells were isolated from freshly resected tumor tissue and immortalized ex vivo using supernatant from the EBV-producing B95/8 marmoset cell line in the presence of cyclosporin A to suppress T- and NK-cell outgrowth. Outgrowth of EBV-transformed B-cell clones typically occurred within several weeks, and clonality was confirmed by immunoglobulin heavy- and light-chain gene rearrangement analysis using BIOMED-2 multiplex PCR protocols.
B-LCL-HROC69 secretes immunoglobulin A (IgA), as determined by isotype-specific ELISA of long-term culture supernatants. In contrast to several IgG-producing TiBc lines established in parallel, IgA derived from HROC69 was not further characterized for tumor cell binding in the initial functional screening assays. Importantly, no spontaneous outgrowth of B-cell cultures occurred in the absence of exogenous EBV, indicating that the immortalization is an in vitro event rather than the consequence of latent EBV infection in vivo. B-LCL-HROC69 therefore represents a monoclonal, antigen-experienced tumor-infiltrating B-cell model suitable for investigating humoral immune responses within the colorectal carcinoma microenvironment and for the potential identification of tumor-associated antigens recognized by locally expanded B-cell clones.

Tissue	Peripheral blood
Disease	Carcinoma

Synonyms

- B-LCL CO69
- Bc HROC69
- TiBcHROC69

CLASSIFICATION

Organism	Human
Sex	Male
Growth properties	Suspension
Biosafety level	2

CHARACTERISTICS

Morphology	Round cells
Cell type	B lymphoblast

IDENTIFIERS

Citation

B-LCL-HROC69 (Cytion catalog number 300864)

NCBI TaxID	9606
Cellosaurus	CVCL_YD53

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EXPRESSION AND SUSCEPTIBILITY

Surface antigens

CD19

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)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

Supplements

Supplement the medium with 10% heat-inactivated FBS

Subculturing

Gently homogenize the cell suspension in the flask by pipetting up and down, then take a representative sample to determine the cell density per ml. Dilute the suspension to achieve a cell concentration of 1 x 10^5 cells/ml with fresh culture medium, and aliquot the adjusted suspension into new flasks for further cultivation.

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.

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

Donor age	62 years

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.

Third-party agreements apply	Yes