

B-LCL-HROC69 Cells | 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.

Organism Human

Tissue Peripheral blood

Disease Carcinoma

Synonyms B-LCL CO69, Bc HROC69, TiBcHROC69

Characteristics

Age 62 years

Gender Male

Ethnicity Caucasian

Morphology Round cells

Cell type B lymphoblast

Growth properties Suspension

B-LCL-HROC69 Cells | 300864**Regulatory Data****Citation** B-LCL-HROC69 (Cytion catalog number 300864)**Biosafety level** 2**NCBI_TaxID** 9606**CellosaurusAccession** CVCL_YD53**Biomolecular Data****Surface antigens** CD19**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** 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 1×10^5 cells/ml with fresh culture medium, and aliquot the adjusted suspension into new flasks for further cultivation.**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°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 $300 \times g$ 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°C , 5% 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°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°C . Storage at -80°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.