Designation: **B-LCL-HROC69**

CLS order number: Cryovial: 300864
Vital: 330864

Origin and General Characteristics	
Depositor:	Michael Linnebacher
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	62 years
Gender:	Male
Tissue:	Blood
Morphology:	Round cells, in clusters
Cell type:	B lymphocyte, transfected using EBV
Growth Properties:	Suspension
Description:	This is one in a series of cell lines which have been established by PD Dr. Michael Linnebacher from the blood of patients suffering from CRC since 2006.
Culture Conditions and Handling	
Culture Medium:	RPMI 1640 medium supplemented with L-glutamine and 10% fetal bovine serum.
Subculturing:	Take an aliquot of the cell suspension to determine the cell concentration, then carefully resuspend the cells and dispense into new flasks which contain fresh medium.
Split Ratio:	Inoculate the fresh medium with $5x10^5$ cells /ml
Fluid Renewal:	1 to 2 times weekly
Doubling time:	n.d.
Freeze Medium:	CM-ACF (CLS order number 800650, 50ml)
Freezing recovery:	Normal
Sterility:	Mycoplasma specific PCR: negative; Mycoplasma specific PlasmoTest: negative.
Biosafety Level:	2
Special Features of the Cell Line	
Viruses:	Free of human pathogenic viruses SV40, JC/BK, HBV, HCV, and HIV. Contains EBV.
Cell Marker:	$CD19^+$, $CD20^+$, $CD23^+$, $CD27^+$, $CD80^+$, $CD83^+$, $CD138^+$, MHC I^+, MHC II^+.
Related Cell Lines:	**HROC69**: CLS Cat.-no. **300828**, cryovial. **HROC69 T0 M2**: CLS Cat.-no. **300829**, cryovial, **330829**, proliferating culture. **HROC69SE**: CLS Cat.-no. **300893**, Set comprising all three related cell lines.

References:

Maletzki C, Jahnke A, Ostwald C, Klar E, Prall F, et al. (2012) Ex-vivo Clonally Expanded B Lymphocytes Infiltrating Colorectal Carcinoma Are of Mature Immunophenotype and Produce Functional IgG. PLoS ONE 7(2): e32639. doi:10.1371/journal.pone.0032639

Recommendations for handling of suspension cell cultures following delivery

Cryopreserved cells

If immediate culturing is not intended, the cryovial(s) must be stored in liquid nitrogen (-196°C) or at least at -80°C after arrival.

If immediate culturing is intended, please follow these instructions:

Quickly thaw by rapid agitation in a 37°C water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. Note: A small ice clump should still remain and the vial should still be cold.

From now on, all operations should be carried out under aseptic conditions.

Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300xg for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later.

Resuspend the cells carefully in 10ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the Subculture section.

Proliferating Cultures

The cell culture flasks are completely filled with cell culture medium to prevent loss of cells during transit. Remove the entire medium except for a sufficient volume to cover the floor of the flask. Incubate at 37°C for 24 hrs.

Sometimes the cultures are handled roughly during transit, and most of the cells detach and float in the culture medium. If this has occurred remove the entire content of the flask and centrifuge at 300x g for 5 minutes. Take off the supernatant, resuspend the cells in 10 ml of culture medium and transfer the entire cell suspension into cell culture flasks of suitable size (do not seed in more than 1T75 flask).

Safety precautions for frozen cell lines

If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed:

- Protective gloves and clothing should be used and a facemask or safety goggles must be worn when storing and/or thawing the cryovial.
- The removal of a cryovial from liquid nitrogen can result in the explosion of the cryovial creating flying fragments.

References: Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.