Sigma-Aldrich®

Data Sheet

721.221 Human HLA Negative B-Lymphoblastoid Cell Line

Cancer Cell Line

Cat. # SCC275

Pack size: ≥1x10^6 viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Background

The human leukocyte antigen (HLA) gene complex encodes the major histocompatibility complex (MHC) proteins, key regulators of the human immune system. MHC proteins carry antigenic determinants for cell-surface recognition by T cells, and allow for immune autorecognition.[1] The diversity of antigens provides a challenge for parsing individual T-cell activators and specific immune responses, and the ability to manipulate specific determinants is a paramount factor for understanding autoimmune diseases that arise from MHC dysfunction.

The 721.221 human HLA-negative B cell lymphoblastoid cell line is a well-established model for immune activation and HLA expression. The absence of class I HLA expression in 721.221 cells allows for functional analysis of individual class I antigens via transformation with individual HLA genes.[2] HLA genes are ectopically expressed at quantitatively normal levels in 721.21 cells.[2] 721.221 cells express the B-cell marker CD19[4] and several NK activator ligands including CD48, CD80, and CD86.[5] The unique characteristics of the 721.221 cell line make it a highly versatile system for immunology research.

Source

The 721.221 cell line was derived from 721 lymphoblastoid cell line exposed to gamma radiation, inducing null mutations in endogenous HLA-A, B, and C class I antigen genes.[1]

Short Tandem Repeat (STR Profile)

D3S1358:	14, 16	D13S317:	11, 12
D7S820:	10, 11	D16S539:	12
vWA:	17	TH01:	9.3
FGA:	23	TPOX:	8, 12
D8S1179:	10, 14	CSF1PO:	11, 12
D21S11:	28	Amelogenin:	X
D18S51:	12, 13	Penta D:	11, 14
D5S818:	12	Penta E:	5, 12

Cancer cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

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Quality Control Testing

- 721.221 Human HLA negative B-lymphoblastoid cells are verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services
- Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

721.221 Human HLA negative B-lymphoblastoid cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

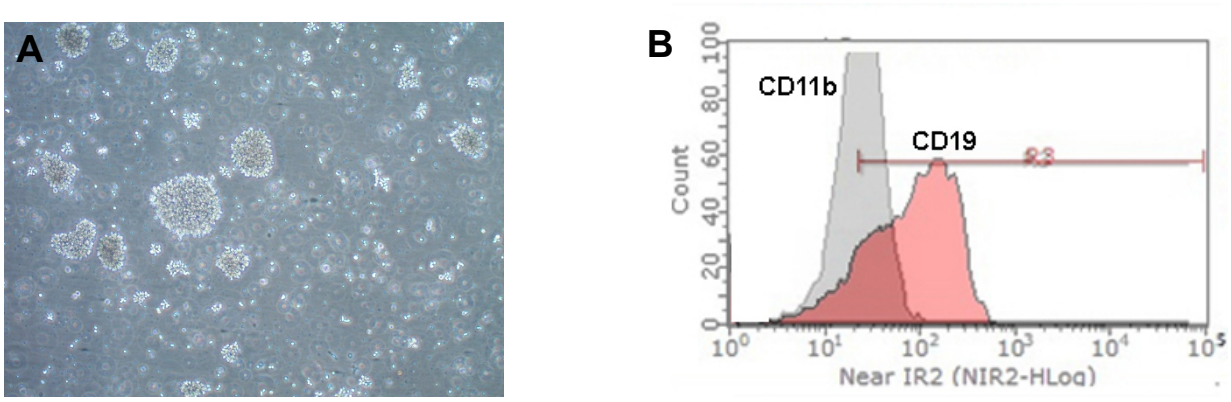

Figure 1. Bright-field image of 721.221 cells two days (A) after thaw. Cells grow as clumpy suspension cells. 721.221 cells are positive for the naïve B-cell marker CD19 (red, BD Pharmingen 561743) and negative for CD11b (gray, Sigma MABF512)

Protocols

Note: 721.221 Human HLA Negative B-lymphoblastoid cells grow as suspension cells and thus do not require enzymatic detachment. Cells may grow as clumpy suspension cells. Passage when the cell density reaches 1–1.5 million cells/mL. During passaging, break up clumps with repeated pipetting. Optimal plating density should be ~200,000 - 250,000 cells/mL. The cells should not be grown at excessively high densities.

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in 721.221 Expansion Medium comprising RPMI-1640 with 25 mM HEPES & L-Glutamine (Sigma SLM-140-B), 1X non-essential amino acids (Sigma TMS-001-C), 1 mM sodium pyruvate (Sigma S8636) and 20% heat-inactivated FBS (Sigma ES-009-B).
2. Remove the vial of frozen 721.221 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.

4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of 721.221 Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of 721.221 Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2. 721.221 suspension cells require media replenishment every 2-3 days. Passage cells when the cell density is at 1 -1.5 million cells/mL.

    Note: Cells may grow as clumpy suspension cells. During passaging, break up clumps with repeated pipetting.
12. Cells are typically plated at a density of 200,000 - 250,000 cells/mL

Cryopreservation of the Cells

721.221 Human HLA Negative B-Lymphoblastoid cells may be frozen in 721.221 Expansion Medium supplemented with 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Simpson E. *Immunol Suppl.* 1988; 1:27-30.
2. Shimizu Y, DeMars R. *J Immunol.* 1989; 142(9):3320-3328.
3. Lisovsky I, Isitman G, Bruneau J, Bernard NF. *J Leukoc Biol* 2015; 97(4):761-767.
4. DeBell KE, Simhadri VR, Mariano JL, Borrego F. *BMC Immunol* 2012; 13:23.
5. Tremblay-McLean A, Coenraads S, Kiani Z, Dupuy FP, Bernard NF. *BMC Immunol* 2019; 20(1):8

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