J14 Human SLP-76-Deficient Jurkat Cell Line

Cell Line

Cat. # SCC190

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.

Data Sheet

Background

T-cell-receptor signaling has a critical role in adaptive immune responses and has been extensively studied as a general model for complex cellular responses.[1] T-cell-receptor signaling to downstream pathways is mediated by non-receptor protein tyrosine kinases (PTKs), which interact with substrate proteins in a complex network.

J14 cells are a unique Jurkat-derived human T cell line that lacks expression of SLP-76 (SH2 domain–containing leukocyte protein of 76 kilodaltons), an adaptor protein and PTK substrate expressed in hematopoietic cells.[2] SLP-76 together with the scaffolding protein LAT act as a hub for various signaling effectors.[1] Except for SLP-76, the expression profile of J14 cells encompasses all necessary components of downstream pathways including those required for IL-2 cytokine induction,[3] making the J14 cell line ideal for mutational analysis and introduction of reporter constructs. J14 cells are a unique cell line that has proven particularly valuable for elucidating cellular signaling mechanisms and interaction network biology.

Source

J14 cells are a clonal derivative of Jurkat, a T cell line derived from bone marrow of a 14-year old male.[4] Jurkat cells were transfected with an episomal vector and selected for hygromycin resistance. The transgene was subsequently lost via non-selective growth.[2]

Short tandem repeat (STR) Profile

D3S1358: 15, 17, 18
TH01: 6, 9.3
D21S11: 31.2, 32.2, 33.2
D18S51: 13, 21, 22
Penta E: 10, 12
D5S818: 9
D13S317: 8, 14, 15
D7S820: 8, 11
D16S539: 11
CSF1PO: 11, 12
Penta D: 11, 13
vWA: 18, 19, 20
D8S1179: 12, 13, 14, 15
TPOX: 8, 10
FGA: 20, 21
Amelogenin: X

Jurkat and derivative cell lines are known to be MSI-high (microsatellite instability), meaning that there is a high amount of instability. MSI+ cell lines tend to vary at a subset of STR loci. For J14 cells, the following 4 loci, D5S818, D16S539, TH and TPOX are more stable and may be used to monitor the genetic stability of J14 at higher passages. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from rat, mouse, chinese hamster, Golden Syrian hamster, and non-human primate (NHP) as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Storage & Handling

J14 Human SLP-76-Deficient Jurkat cell line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

References

1. *J Cell Sci.* 2009; 122(Pt 9): 1269-1273.
2. *J Biol Chem.* 1995; 270(13): 7029-7032.
3. *Science.* 1998; 281(5375): 413-416.
4. *Int J Cancer.* 1977; 19(5): 621-626.

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Protocols

J14 cells grow as suspension cells and thus do not require enzymatic detachment or dissociation. Passage when the cell density reaches 1–1.5 million cells/mL. 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. J14 Expansion Medium: Cells are thawed and expanded in RPMI-1640 (Sigma Cat. No. R0883) supplemented with 2 mM Glutamine (Cat. No. TMS-002-C) and 5% FBS (Cat. No. ES-009-B).
2. Remove the vial of frozen J14 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 J14 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 – 20 mL of J14 Expansion Medium.
10. Transfer the cell suspension to a T75 flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2. J14 suspension cells require media replenishment every 2-3 days. Passage cells when the cell density is at 1 -1.5 million cells/mL.
12. Cells are typically plated at a density of 200,000 - 250,000 cells/mL

Cryopreservation of Cells

J14 Human SLP-76-Deficient Jurkat Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

Representative Data

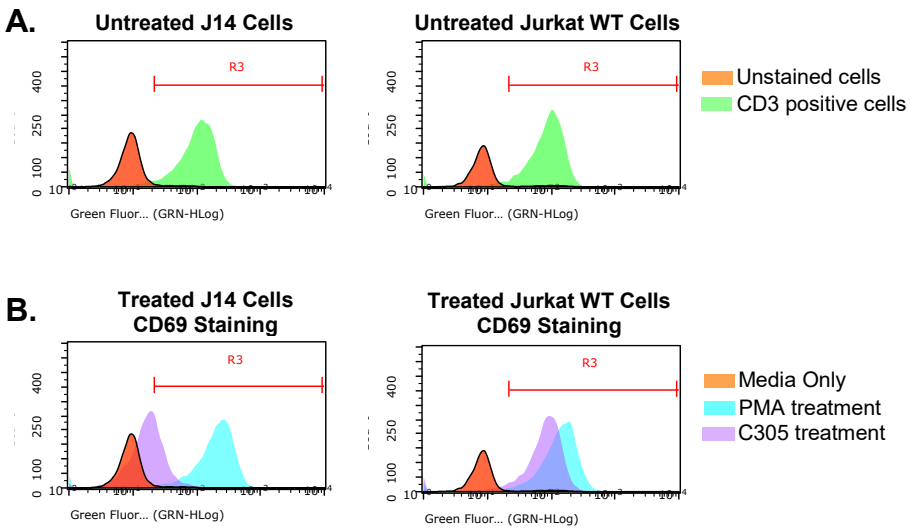

Figure 1. FACs analyses of wild-type and J14 Jurkat cells treated with 50 ng/mL PMA or 0.1 µg/mL anti-T-cell receptor, clone C305 for 14 hours. Both wild-type Jurkat and J14 cells express CD3, a marker of T lymphocytes (A). CD69 expression is induced in J14 and WT Jurkat cells in a Ras-dependent manner following stimulation with anti-TCR or with phorbol myristate acetate (PMA). TCR-inducible gene expression is dependent on SLP-76. With C305 treatment, CD69 is induced only in WT Jurkat and not in J14 cells, indicating that J14 lacks TCR-inducible expression of CD69, despite normal TCR expression and normal PMA-induced up-regulation of CD69 (B).[3]

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