IncuCyte™ Jurkat NucLight™ Red Cells

Catalog number: 4613

Immortalized Human T lymphocyte cells stably expressing nuclear restricted red fluorescent protein.

Presentation, storage and stability

Jurkat NucLight™ Red Cells are supplied as 1 mL cryopreserved vials (1.5 x 10^6 cells/mL in 90% FBS and 10% DMSO) containing a stable population of human T lymphoccyte cells expressing the NucLight™ Red fluorescent protein, restricted to the nucleus. NucLight™ cells should be stored in liquid nitrogen (vapour phase). Do not store at -80°C. When stored as recommended, the NucLight™ cells will be viable for at least 1 year from the date of receipt.

Background and intended use

Parental Jurkat (clone E6.1) cells (ATCC, Cat# TIB-152) were transduced with the IncuCyte™ NucLight™ Red Lentivirus Reagent (Cat# 4476; EF1α, puromycin). 48 hours after transduction, the cell population was grown in complete growth media containing 1 µg/ml puromycin, for 3 to 5 days, to select for cells expressing NucLight™ Red. Following selection the Jukat NucLight™ Red Cells were validated by comparing morphology and chemotactic migration profiles to the parental cell line. The Jurkat NucLight™ cells are certified mycoplasma free and are STR authenticated by ATCC.

Recommended use

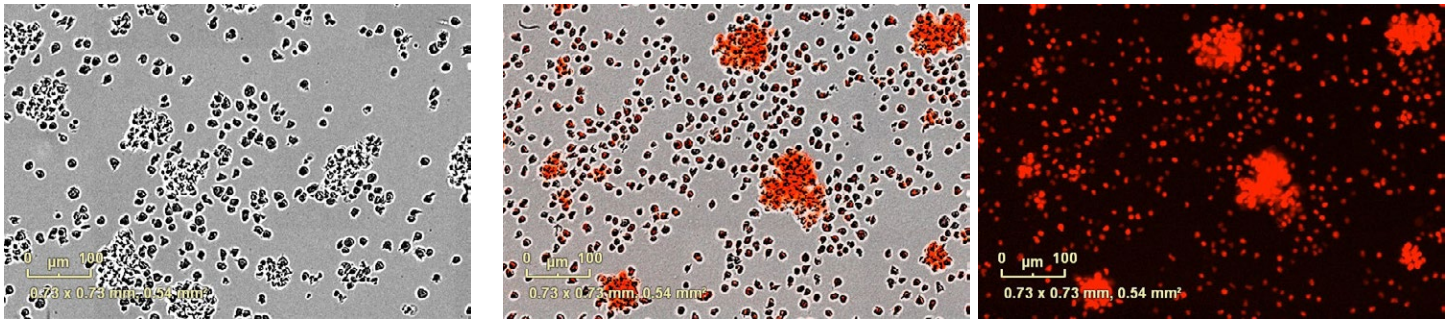
Jurkat NucLight™ Cells should be thawed quickly in a 37°C water bath. Jurkat NucLight™ cells should be maintained at a density of between 0.1-1 x 10^6 cells/ml in growth medium containing 0.5 µg/mL puromycin. These cells are fully validated for use with the IncuCyte ZOOM® live-cell imaging and analysis system and are ideal for use in real-time cell counting studies, co-cultures and chemotaxis assays.

Please see the relevant protocol published on our website:
essenbioscience.com/nuclight

Safety data sheet (SDS) information

The SDS can be found on our website:
essenbioscience.com/nuclight

Parent Jurkat | **Jurkat NucLight™ Red Cells**



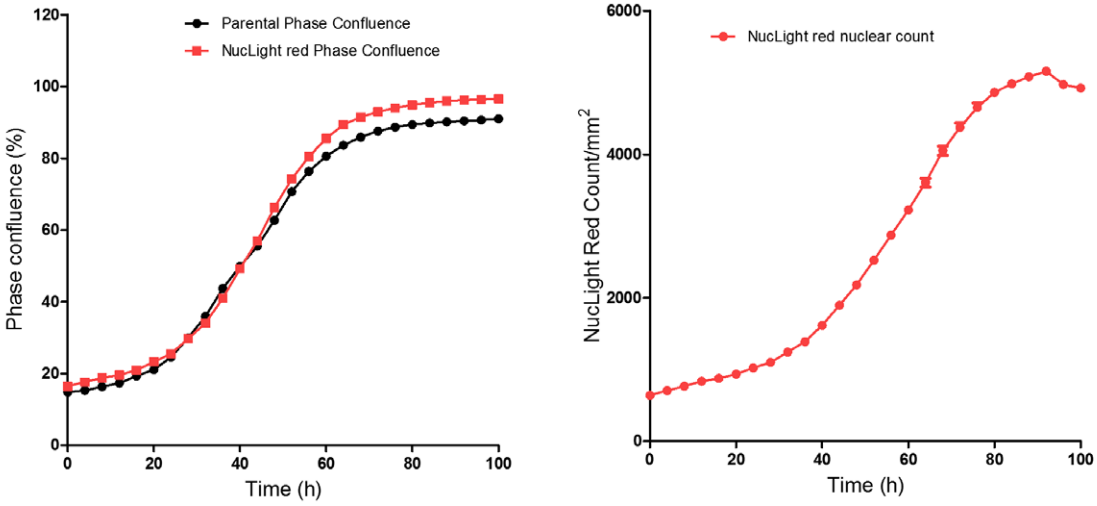


Figure 1. Representative images of Jurkat parent and Jurkat NucLight™ Red cells (fluorescence/phase blend and fluorescence only) and growth characteristics. Cells were seeded in PLO (0.01%) coated flat bottom plates and images were acquired using an IncuCyte ZOOM® system with 10x objective every 4 h. Growth curves were constructed from confluence data for both cell lines and red object count for Jurkat NucLight™ Red cells.

Recommended Media and Components

- RPMI (Cat# 21875 (UK) 11875 (US) Gibco/Life Technologies)
- 10% FBS (Cat# SH30071 Thermo Hyclone)
- 0.5 µg/ml Puromycin (Cat# A11138-03 Gibco/Life Technologies)

Growth Media

- RPMI + 10% FBS + 0.5µg/ml Puromycin

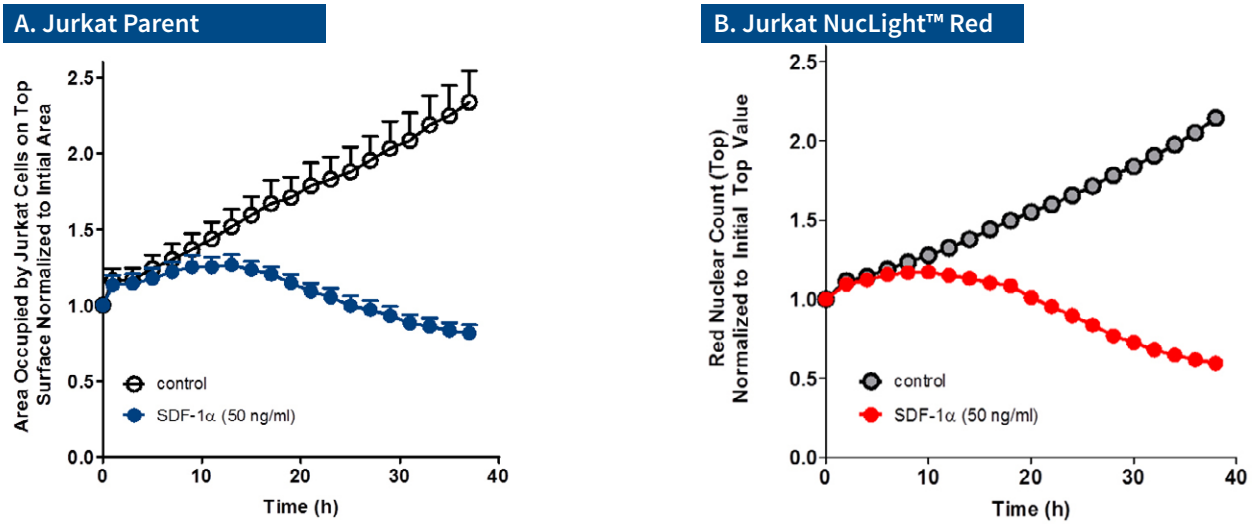


Figure 2. Expression of NucLight™ Red has no impact on the chemotactic profiles of Jurkat cells. (A) Time courses for the directional migration of parental jurkat cells and **(B)** NucLight™ Red cells towards SDF-1α (50ng/ml) are comparable. In both studies, cells were plates at 5,000 cells per well in a 96-well IncuCyte™ ClearView™ plate and images were captured at 1 or 2 hour intervals in an IncuCyte® ZOOM system with 10x objective.

Optimization protocol

Unpacking and Storage Instructions

1. Check all containers and vials for leakage or breakage.
2. Remove the frozen vial from the dry ice packaging and transfer immediately to liquid nitrogen storage until ready for use.

Thawing and Culturing Cells

Jurkat NucLight™ Red Cells are supplied as non-adherent, immortalized T lympocyte cells and should be maintained at a cell density of between 0.1-1 x 10^6 cells/ml, do not allow the cell density to exceed 3 x 10^6 cells/ml. Cell doubling time is approximately 20 hours. We recommend thawing one vial of Jurkat NucLight™ Red cells into one T25 flask with 6 mL of media.

1. Remove the vial of Jurkat NucLight™ Red cells from liquid nitrogen storage.
2. Thaw the vial by gentle agitation in a 37°C water bath. Be careful not to submerge entire vial to avoid contamination. This process should take no more than 2 minutes. Remove the vial when only a tiny ice crystal remains.
3. Wipe the vial with 70% ethanol.
4. Transfer the vial contents to a centrifuge tube containing 9 mL of growth media and centrifuge at 125 x g for 5 min.
5. Remove the supernatant taking care not to disturb the cell pellet.
6. Resuspend the cells in 6 mL of growth media and add to a T25 flask

   Note: To maintain expression of the NucLight™ Red label, we recommend culturing the cells in growth media containing 0.5 µg/ml puromycin. Puromycin can be removed for experiment/assay set-up.
7. Incubate the cells at 37°C incubator, 5% CO_2.
8. Cultures can be maintained by the addition or replacement of fresh growth medium.

Licenses and Warranty