IncuCyte™ THP-1 NucLight™ Red Cells

Catalog number: 4612

Human monocytes stably expressing nuclear restricted red fluorescent protein.

Presentation, storage and stability

THP-1 NucLight™ Red Cells are supplied as 1 mL cryopreserved vials (1 x 10^6 cells/mL in 90% FBS and 10% DMSO) containing a stable population of human monocytic cells expressing the NucLight™ Red fluorescent protein, restricted to the nucleus. NucLight™ cells should be stored in liquid nitrogen. 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 THP-1 cells (ATCC, Cat# TIB-202) 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 2 µg/ml puromycin, for 3 to 5 days, to select for cells expressing NucLight™ Red. Following selection the THP-1 NucLight™ Red Cells were validated by comparing morphology and chemotactic migration profiles to the parental cell line. The THP-1 NucLight™ cells can be differentiated into macrophage-like cells while maintaining nuclear restricted fluorescence and are certified mycoplasma free and are STR authenticated by ATCC.

Recommended use

THP-1 NucLight™ Cells should be thawed quickly in a 37°C water bath. THP-1 NucLight cells should be maintained at a density of between 2 to 8 x 10^5 cells/mL in growth medium containing 0.5 µg/mL puromycin. The THP-1 cells can be differentiated in to macrophage-like cells by treating with 5 ng/mL PMA (Phorbol 12-myristate 13-acetate) for 48 hours. 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

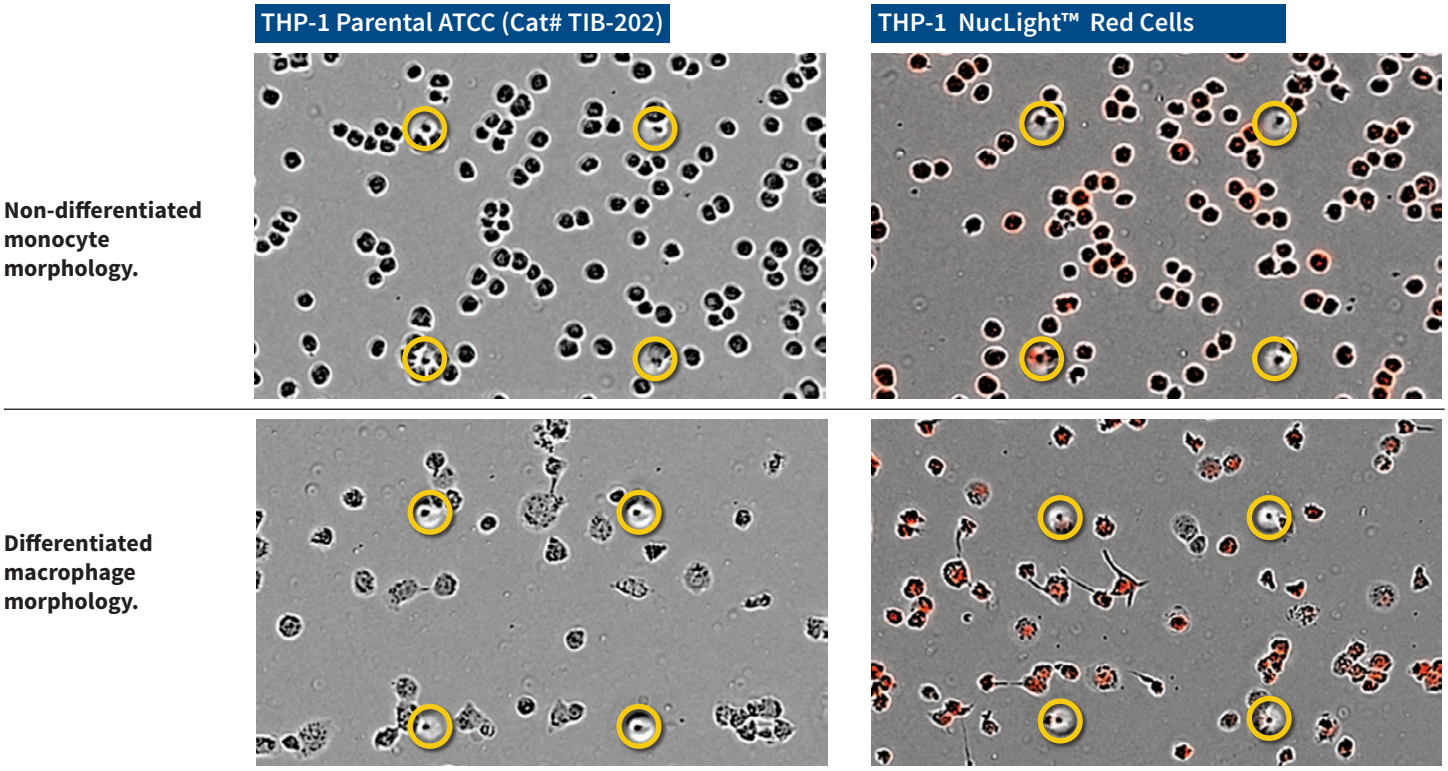

Figure 1. Representative images of THP-1 parental and THP-1 NucLight™ Red cells showing the non-differentiated, monocyte, and differentiated macrophage-like morphologies following treatment with PMA (5 ng/mL) for 48 hours. Note the nuclear restricted expression of red fluorescent protein and the healthy cell morphology. Cells were seeded into an IncuCyte™ ClearView™ Cell Migration Plate (membrane pores circled orange) and images were acquired using and an IncuCyte ZOOM® system with 10x objective.

Recommended Media and Components

- RPMI (Cat# 11875-085 Life Technologies)
- 10% FBS (Cat# SH30071 Thermo Hyclone)
- 0.05 mM ß –mercaptoethanol (Cat# 21985-023 Thermo)
- 1% Pen/Strep (Cat# 15140 Gibco/Life Technologies)
- 0.5 µg/ml Puromycin (Cat# A11138-03 Gibco/Life Technologies)
- 5 ng/mL Phorbol 12-myristate 13-acetate (PMA) (Cat# P1585) – optional

Growth Media

- RPMI + 10% FBS + 0.1% ß-ME + 0.5µg/ml Puromycin + 1% Pen/Strep

Differentiation Media

- RPMI + 10% FBS + 0.1% 2 ß-ME + 5 ng/mL PMA+ 1% Pen/Strep

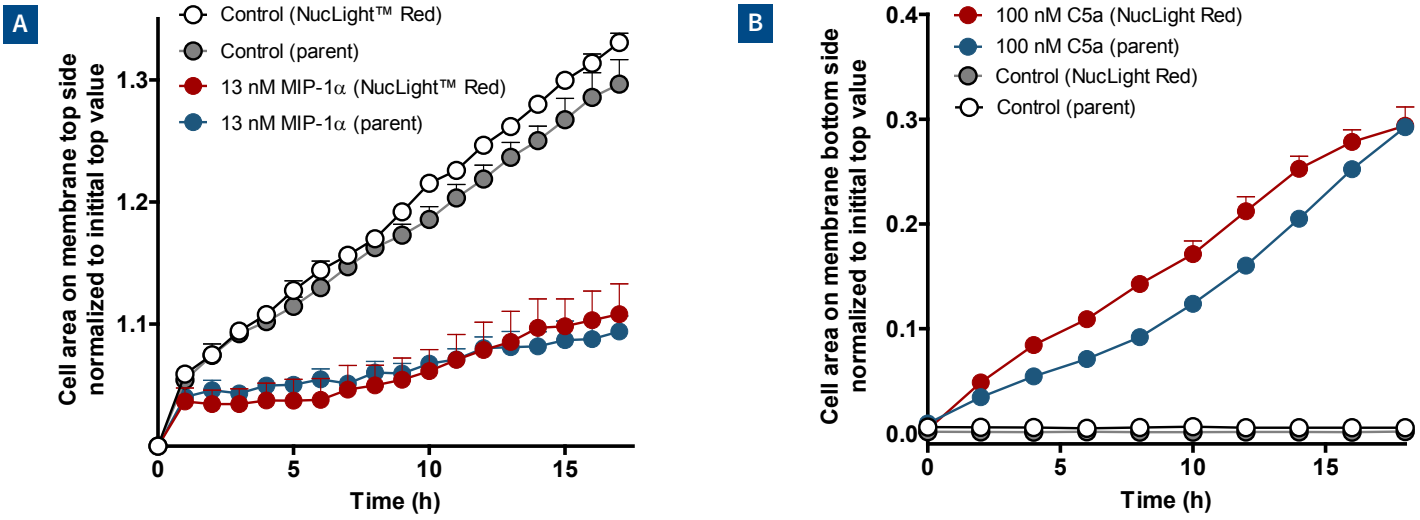

Figure 2.Expression of NucLight™ Red has no impact on the chemotactic profiles of non-differentiated or differentiated THP-1 cells. (A) Time courses for the directional migration of parental and THP-1 NucLight™ Red cells. Chemotaxis profiles of non-differentiated THP-1 NucLight™ Red cells towards the macrophage inflammatory protein MIP-1α (13 nM) were comparable to parental THP-1 cells. **(B)** Following differentiation to a macrophage-like morphology (48 hour treatment with 5 ng/mL PMA) chemotactic profiles towards C5a (100nM) were also shown to be comparable between parental and THP-1 NucLight™ Red cells.

In both studies, cells were plated 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.

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.

Product	Cat No.	Amount
IncuCyte™ A549 NucLight™ Red	4491	1 x 10^6 cells /vial
IncuCyte™ A549 NucLight™ Green	4492	1 x 10^6 cells /vial
IncuCyte™ HeLa NucLight™ Red	4489	1 x 10^6 cells /vial
IncuCyte™ HeLa NucLight™ Green	4490	1 x 10^6 cells /vial
IncuCyte™ HT-1080 NucLight™ Red	4485	1 x 10^6 cells /vial
IncuCyte™ HT-1080 NucLight™ Green	4486	1 x 10^6 cells /vial
IncuCyte™ MCF7 NucLight™ Green	4528	1 x 10^6 cells /vial

Product	Cat No.	Amount
IncuCyte™ MCF7 NucLight™ Red Cells	4524	1 x 10^6 cells /vial
IncuCyte™ MDA-MB-231 NucLight™ Red	4487	1 x 10^6 cells /vial
IncuCyte™ MDA-MB-231 NucLight™ Green	4488	1 x 10^6 cells /vial
IncuCyte™ THP-1 NucLight™ Red Cells	4612	1 x 10^6 cells /vial
IncuCyte™ Jurkat NucLight™ Red Cells	4613	1.5x10^6 cells/vial
IncuCyte™ Neuro-2a NucLight™ Green	4511	4 x 10^5 cells/vial
IncuCyte™ Neuro-2a NucLight™ Red	4512	4 x 10^5 cells/vial

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

THP-1 NucLight™ Red Cells are supplied as non-adherent, immortalized monocytic cells and should be maintained at a cell density of between 2 to 8 x 10^5 cells/mL. Cell doubling time is approximately 48 hours. We recommend thawing one vial of THP-1 NucLight™ Red cells into one T25 flask with 6 mL of media.

1. Remove the vial of THP-1 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