DATA EXAMPLES

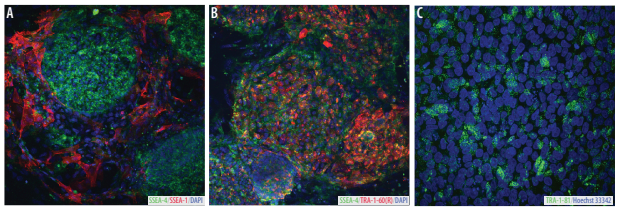

Figure 1: Verification of Pluripotency in Human Induced Pluripotent Stem Cells. (A,B) iPS2 induced pluripotent stem cells grown on irradiated mouse embryonic fibroblasts (R&D Systems, Catalog # PSC001), and **(C)** JOY1 human induced pluripotent stem cells grown in mTeSR®1 under feeder-free conditions, were stained using the antibodies included in the Human Pluripotent Stem Cell Live Cell Imaging Kit. **(A)** iPS2 cells were stained with the NL493-conjugated SSEA-4 (green) and the NL557-conjugated SSEA-1 (red) antibodies. **(B)** iPS2 cells were stained with the NL493-conjugated SSEA-4 (green) and the NL557-conjugated TRA-1-60(R) (red) antibodies. **(C)** JOY1 cells were stained with the NL493-conjugated TRA-1-81 (green) antibody. All cells were counterstained with Hoechst 33342 (blue). The cells are positive for the stem cell markers SSEA-4, TRA-1-60(R), or TRA-1-81 and are negative for SSEA-1, suggesting that these colonies primarily contain undifferentiated human pluripotent stem cells.

DATA EXAMPLES *CONTINUED*

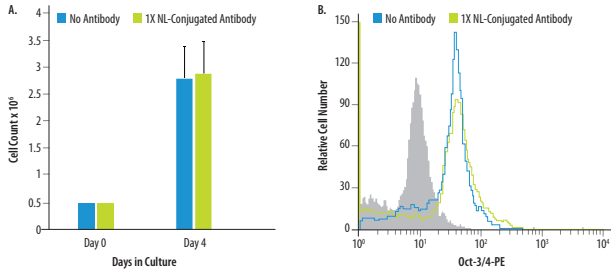

Figure 2: Analysis of Human Embryonic Stem Cell Growth and Stemness Post-staining. (A) BG01V human embryonic stem cells were plated in triplicate in 6 well plates at 0.5 x 10^6 cells per well on Day 0. On Day 1, cells were stained with (green bars) or without (blue bars) 1X NL-conjugated antibody. On Day 4, cells were harvested and counted using a hemocytometer. There was no effect of SSEA-4, TRA-1-60(R), or SSEA-1 staining on cell proliferation. Error bars indicate standard deviation. **(B)** BG01V human embryonic stem cells were stained with (green histogram) or without (blue histogram) 1X NL-conjugated antibody and cultured for 3 days. Cells were then harvested and stained using the pluripotent marker PE-conjugated Rat Anti-Human/Mouse Oct-3/4 Monoclonal Antibody (R&D Systems®, Catalog # IC1759P; open histogram) or a Rat IgG2B PE-conjugated Isotype Control (R&D Systems®, Catalog # IC013P; filled histogram). Staining with SSEA-4, TRA-1-60(R), or SSEA-1 had no effect on stemness as analyzed by Oct-3/4 expression. Stemness post-staining was also verified by SSEA-4 and SSEA-1 expression (data not shown).

GloLIVE™

Human Pluripotent Stem Cell Live Cell Imaging Kit

Catalog Number: SC023B
Size: 25 Tests

PRODUCT DESCRIPTION

This kit contains four stem cell marker antibodies conjugated to NorthernLights™ fluorochromes that can be used for single-step, quick, and direct immunocytochemical staining of live unfixed human pluripotent stem cells (including embryonic stem cells and induced pluripotent stem cells). The cells are able to continue in culture after staining without affecting cell proliferation or stemness. Each antibody is supplied as 250 µL of a 50X stock in PBS. A 1X antibody solution has an endotoxin level of ≤ 5 EU/mL.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:
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MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C **in the dark.**
Use within 6 months of receipt.

PART	PART #	DESCRIPTION
anti-h/mSSEA-4 NL493 Conjugated Mouse IgG_3 (50X)	893871	250 uL of a 50X stock in PBS.
anti-h/mSSEA-1 NL557 Conjugated Mouse IgM (50X)	893872	250 uL of a 50X stock in PBS.
anti-hTRA-1-60 (R) NL557 Conjugated Mouse IgM (50X)	893874	250 uL of a 50X stock in PBS.
anti-hTRA-1-81 NL493 Conjugated Mouse IgM (50X)	968392	250 uL of a 50X stock in PBS.

SPECTRAL CHARACTERISTICS

The spectral characteristics of each of the fluorochromes used are described below.

Fluorochrome	Absorption Maximum (nm)	Emission Maximum (nm)
NL557	559	575
NL493	493	514

PRECAUTION

Use aseptic technique and sterile culturing conditions to prevent contamination.

IMMUNOCYTOCHEMISTRY PROCEDURE

These antibodies have been tested for immunocytochemistry using human induced pluripotent stem cells grown either on irradiated mouse embryonic fibroblast feeder cells or in feeder-free conditions. Each antibody is supplied as a 50X stock; enough for 25 assays when used in 500 µL staining volume per assay.

To ensure sterility of cultures, all steps should be performed under sterile conditions.

1. Dilute the desired antibody to a 1X concentration (1:50) in appropriate culture media.
2. Remove the media from the cells. Gently add fresh media containing 1X antibody, and return the cells to the incubator for 30 minutes.
3. Remove the antibody-containing media, rinse the cells once with fresh media, and re-feed with fresh media.
4. The cells can be visualized at this point or continued for further culture if desired.

   Note: *Culture with antibodies does not appear to affect cell proliferation or stemness as assayed by proliferation curves for 3 days post-antibody incubation and expression levels of SSEA-4 and Oct-3/4.*

NOTES