

### PRODUCT DESCRIPTION

This kit contains three stem cell marker antibodies conjugated to NorthernLights™ fluorochromes that can be used for single-step, quick, 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.

### MATERIALS PROVIDED & STORAGE

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 <sub>3</sub> (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.

### 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

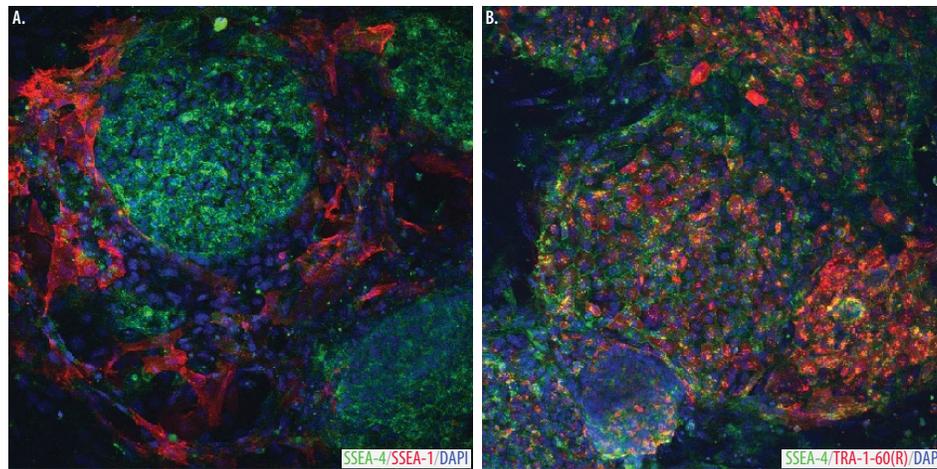
These antibodies have been tested for immunocytochemistry using human induced pluripotent stem cells grown either on irradiated mouse embryonic fibroblast feeder cells or in feeder-free conditions. Each antibody is supplied as a 50X stock; enough for 25 assays when used in 500 µL staining volume per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

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

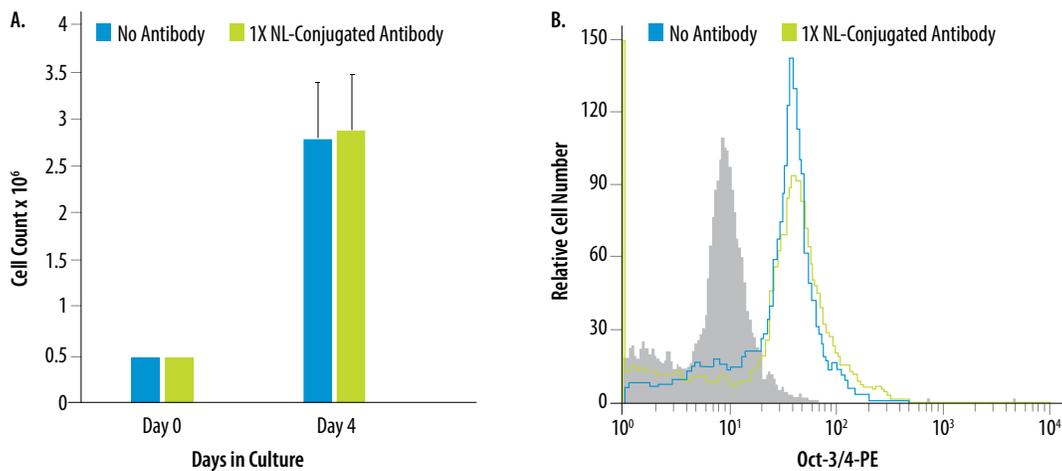
1. Dilute the desired antibody to a 1X concentration (1:50) in appropriate culture media.
2. Remove the media from the cells. Gently add fresh media containing 1X antibody, and return the cells to the incubator for 30 minutes.
3. Remove the antibody-containing media, rinse the cells once with fresh media, 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.

## DATA EXAMPLES



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



**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  $0.5 \times 10^6$  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).

BG01V cells are licensed from ViaCyte, Inc.