Boost your stem cell antibody arsenal

Monoclonal antibodies for studying human pluripotent stem cells.

Stem cells are undifferentiated cells that have the capacity both to self-renew through mitosis and to differentiate into specialized cell types such as neurons or muscle cells. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are pluripotent stem cells (hPSCs) that can divide for a long period of time in culture and have the potential to differentiate into any cell type found in the human body [1]. Both differentiated and undifferentiated stem cells are identified and characterized using primary antibodies to key targets, which are detected by fluorescence-based methods such as immunofluorescence imaging and flow cytometry. hPSCs are commonly identified using monoclonal antibodies (mAbs) that recognize cell-surface proteins, including SSEA3, SSEA4, TRA-1-60, TRA-1-81, and GCTM-1, as well as the intracellular transcription factor OCT4. The study and application of stem cells will be enhanced by the availability of well-characterized hPSC-specific mAbs detecting cell-surface epitopes.

Cell-surface markers for pluripotent stem cells

A research team from CSIRO in Australia has recently described the generation of a set of mAbs that recognize cell-surface proteins present on hESCs and hiPSCs [2]. These cell-surface proteins were previously identified in a study examining spontaneously differentiating hPSCs that were also rapidly losing immunoreactivity to two cell-surface markers (GCTM-2 and CD9) associated with human pluripotency [3]. Bioinformatic analysis of the gene signature of these cells identified 88 cell-surface proteins, most of which had not been previously associated with undifferentiated hPSCs [3]. Here we report on the characterization of four mAbs (CSTEM26, CSTEM27, CSTEM28, and CSTEM29 clones) capable of detecting these cell-surface markers on live hPSCs. An interview with Andrew Laslett, PhD, who was part of the team that developed and characterized these antibodies, is available at **thermofisher.com/stemcellabs**.

Characterizing the stem cell antibodies

Antibodies against CUB domain–containing protein 1 (CDCP1 or CD318, clone CSTEM26), platelet F11 receptor (F11R or CD321, clone CSTEM27), desmoglein 2 (DSG2, clone CSTEM28), and P-cadherin (cadherin 3 or CDH3, clone CSTEM29) were shown to be highly specific for their respective target cell-surface proteins by ELISA (data not shown), indirect immunofluorescence of fixed hESCs (Figure 1), and flow cytometry using live hPSCs (Figure 2) [2]. The specificity of the antibodies for their target cells was further verified using flow cytometry to detect their coexpression with the transcription factor OCT4 in fixed cells (Figure 3) and the cell-surface proteins TRA-1-60 and SSEA-4 in live cells (data not shown).

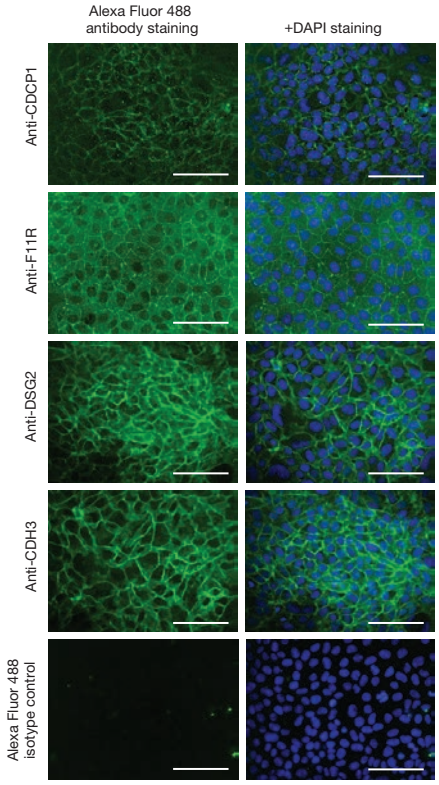

Figure 1. Immunostaining of undifferentiated MEL1 human embryonic stem cells. Undifferentiated MEL1 human embryonic stem cells (hESCs) cultured on a feeder layer of mouse embryonic fibroblasts in human pluripotent stem cell (hPSC) medium for 4–7 days were fixed in cold 100% ethanol and surface-labeled with anti-CDCP1 (Cat. No. 14-3189-80), anti-F11R (Cat. No. 14-3219-80), anti-DSG2 (Cat. No. 14-9159-80), or anti-CDH3 (Cat. No. 14-2237-80) monoclonal antibodies (or the corresponding isotype control) in conjunction with Invitrogen™ Alexa Fluor™ 488 secondary antibodies (green), counterstained with DAPI nucleic acid stain (blue), and imaged by fluorescence microscopy. Scale bars = 100 µm.

Additional studies have demonstrated that these new antibodies can be used to sort and enrich hPSCs for use with transcriptome or differentiation studies. Cells with high expression of the target proteins were sorted by fluorescence-activated cell sorting (FACS) and re-plated to form self-renewing cell colonies that contain high percentages of OCT4-positive hPSCs (data not shown) [2].

Fluorescent conjugates of the antibodies against CDCP1, F11R, and CDH3 were shown to detect a high percentage of the TRA-1-60–positive cells from a mixture of hiPSCs and the murine C2C12 cell line, demonstrating their use for identifying hiPSCs in mixed-cell populations. These antibodies also appear to have uses not directly associated with pluripotency. For example, anti-CDCP1, anti-F11R, and anti-DSG2 were shown to detect antigen expression on human breast epithelial and stromal subpopulations by flow cytometry (data not shown) [2], suggesting potential as breast cancer biomarkers.

Selection tools for stem cell antibodies

Characterization of stem cells is a critical step in stem cell research. The mAbs reported here—exclusively available through the Invitrogen™ antibody portfolio—add to our set of stem cell antibodies for detecting and purifying subpopulations of stem cells, for enriching hPSCs before differentiation, and for conducting quantitative comparisons of hPSC lines. No matter which detection platform you use—flow cytometry, microscopy, western blotting, ELISA, or other—the collection of over 40,000 antibodies from Thermo Fisher Scientific provides you with choices compatible with your experimental design. Visit **thermofisher.com/antibodiesbp76**, where you will find an antibody search tool that allows you to filter by target, antibody type, application, and host species. ■

Antibody target	Clone	Format	Quantity	Cat. No.
CD318 (CDCP1)	CSTEM26	Unconjugated	25 µg	14-3189-80
			100 µg	14-3189-82
		PE	25 tests	12-3189-41
			100 tests	12-3189-42
		Alexa Fluor 647	25 tests	51-3189-41
			100 tests	51-3189-42
CD321 (F11R)	CSTEM27	Unconjugated	25 µg	14-3219-80
			100 µg	14-3219-82
		PE	25 tests	12-3219-41
			100 tests	12-3219-42
		Alexa Fluor 488	25 tests	53-3219-41
			100 tests	53-3219-42
Desmoglein 2 (DSG2)	CSTEM28	Unconjugated	25 µg	14-9159-80
			100 µg	14-9159-82
		PE	25 tests	12-9159-41
			100 tests	12-9159-42
		Alexa Fluor 488	25 µg	53-9159-80
			100 µg	53-9159-82
P-cadherin (CDH3)	CSTEM29	Unconjugated	25 µg	14-2237-80
			100 µg	14-2237-82
		APC	25 tests	17-2237-41
			100 tests	17-2237-42

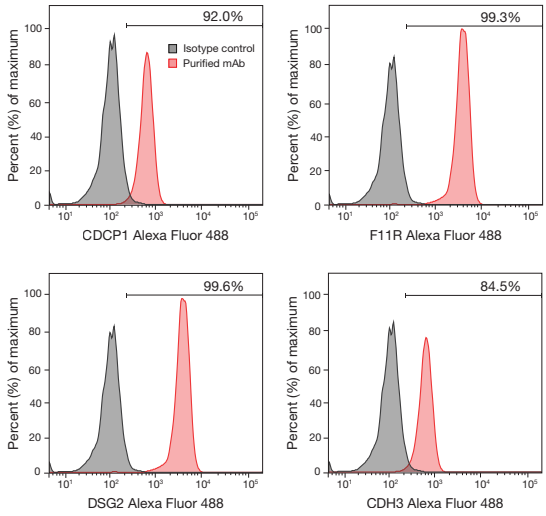

Figure 2. Immunostaining of undifferentiated human pluripotent stem cells using stem cell–specific cell-surface markers. Live undifferentiated WA09 human embryonic stem cells (hESCs) were immunolabeled with anti-CDCP1, anti-F11R, anti-DSG2, anti-CDH3 monoclonal antibodies (pink peak) or the corresponding isotype control (gray peak), detected with Invitrogen™ Alexa Fluor™ 488 secondary antibodies, and analyzed by flow cytometry. The histograms show that a high percentage of total live WA09 hESCs express the stem cell–specific antigens.

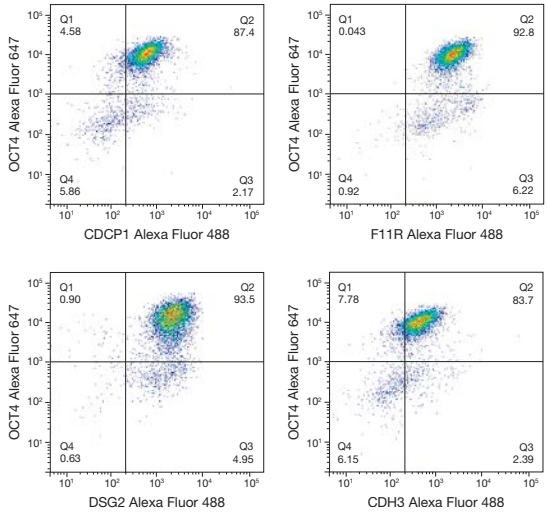

Figure 3. Coexpression of stem cell–specific cell-surface proteins and the transcription factor OCT4 on human embryonic stem cells. Flow cytometry dot plots show high coexpression of human pluripotent stem cell (hPSC)–specific cell-surface proteins (detected with CSTEM clones and Invitrogen™ Alexa Fluor™ 488 secondary antibodies) and OCT4 (detected with anti-OCT4 antibody and Invitrogen™ Alexa Fluor™ 647 secondary antibody) following sequential live- and fixed-cell immunolabeling of WA09 human embryonic stem cells (hESCs).

References

1. Laslett AL, Filipczyk AA, Pera MF (2003) *Trends Cardiovasc Med* 13:295–301.
2. O'Brien CM, Chy HS, Zhou Q et al. (2017) *Stem Cells* 35:626–640.
3. Kolle G, Ho M, Zhou Q et al. (2009) *Stem Cells* 27:2446–2456.