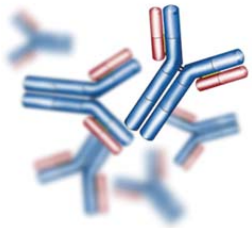
System Biosciences



Human ES/iPS Cell Characterization Kits

Cat. # SABxxx-1

User Manual

See boxes for proper storage of the kit components upon receipt

Contents

I. Introduction and Background

A. Pluripotency

Pluripotency refers to the potential of cell to differentiate into any of the three germ layers, endoderm (interior stomach lining, gastrointestinal tract, the lungs), mesoderm (muscle, bone, blood, urogenital), or ectoderm (epidermal tissues and nervous system). Pluripotency is a property shared by embryonic stem (ES) cells, embryonic germ (EG) cells and embryonic carcinoma (EC) cells. Like ES cells, bona fide induced pluripotent stem (iPS) cells are also pluripotent. Validation of pluripotency is an important aspect of stem cell and iPS cell research.

B. Human ES/iPS Cell Characterization Kit

SBI's Human ES/iPS Cell Characterization Kit contains Alkaline Phosphotase Staining Kit and four antibodies of pluripotency markers including transcription factors Nanog and Oct4, and cell surface antigens SSEA3 and TRA-1-60.

Alkaline phosphatase (AP) is a universal pluripotent marker for all types of pluripotent stem cells including embryonic stem (ES) cells, embryonic germ (EG) cells, and induced pluripotent stem (iPS) cells. Alkaline phosphatase (AP) is a hydrolase enzyme responsible for dephosphorylating molecules such as nucleotides and proteins under alkaline conditions. AP staining is a histochemical assay for cells grown in tissue culture wells or dishes.

Nanog is a transcription regulator involved in inner cell mass and embryonic stem (ES) cell proliferation and self-renewal. It imposes pluripotency on ES cells and prevents their differentiation towards extraembryonic endoderm and trophectoderm lineages. Nanog has been reported to block bone morphogenetic protein-induced mesoderm differentiation of ES cells by physically interacting with SMAD1 and interfering with the recruitment of coactivators to the active SMAD transcription.

Octamer transcription factor-4 (Oct-4) is a homeodomain transcription factor in the POU transcription factor family that

specifically binds to the octamer motif (5'-ATTTCAT-3'). It is critical for early embryogenesis and for embryonic stem cell pluripotency, expressed mainly in the developing brain. OCT4 is a master regulator that affects the fate of pluripotent stem cells and germ cell precursors. For instance, the downregulation of Oct-4 triggers embryonic stem cell differentiation, simultaneous with gastrulation. It is also used as a marker for germ cell tumor diagnosis.
TRA-1-60 antibody recognizes an antigen that is associated with a pericellular matrix proteoglycan. TRA-1-60 antibody reacts with a neuraminidase resistant epitope. The epitope for TRA-1-60 is found on the surface of human EC cells, human EG cells and human ES and iPS cells.
SSEA3 (stage-specific embryonic antigen) is a cell surface glycosphingolipid considered an embryonic/pluripotency marker. Human EC and ES cells express SSEA-3 and SSEA-4, and differentiation is accompanied by an upregulation of SSEA-1 and down-regulation of SSEA-3 and SSEA-4.

All the antibodies react with antigens that are expressed in human pluripotent stem cells. No immunoreactivity is seen with murine pluripotent stem cells.

C. Kit Components

1. Alkaline Phosphotase Staining Kit (5 mL + 5 mL)
   (AP Substrate Solution A & B)
   (Cat. No. AP100R-2)
2. Purified Rabbit Monoclonal Nanog Antibody (25 µL)
   (Cat. No. SAB-103A-1)
3. Purified Rabbit Monoclonal Oct4 Antibody (25 µL)
   (Cat. No. SAB-105A-1)
4. Purified Mouse Monoclonal TRA-1-60 Antibody (25 µL)
   (Cat. No. SAB-100A-1)
5. Purified Rat Monoclonal SSEA3 Antibody (25 µL)
   (Cat. No. SAB-102A-1)

D. Additional Materials and Instruments Needed

1. Phosphate Buffered Saline (PBS)
2. Triton X-100
3. 4% Paraformaldehyde in PBS
4. Animal Serum
5. Fluorescence Microscope or Confocal Microscope

E. Storage

Store all components at 4°C until the expiration date.

II. Protocols

The amounts of reagents below are designed for use in 24-well format. It can be scaled up and down according to the plate format and well size.

A. Protocol for AP staining

1. Gently aspirate the culture medium and wash the cells twice with 0.5 ml PBS. Aspirate the wash solution.
2. Add 4% paraformaldehyde 0.5 ml per well. Incubate at room temperature for 5 min. (Do not fix your cells beyond

20 min, as over-fixation may result in the loss of AP activity.)

3. Aspirate the 4% paraformaldehyde solution and wash the fixed cells with PBS twice.
4. Mix AP substrate solution A and solution B at 1:1 ratio at room temperature. (For optimal results, the AP substrate solution should be used within 30 min of preparation.)
5. Aspirate PBS from cells and add freshly prepared AP substrate solution into each well. For a 24-well plate, add 0.4 ml per well.
6. Incubate the cells with substrate at room temperature for 15 to 20 min, protected from light.
7. Stop the reaction by aspirating the substrate solution and rinsing twice with PBS.
8. Cover the cells with PBS to prevent drying out.
9. AP expression results in red color staining on colonies, while the absence of AP expression results in no staining.
10. Observe the red color stained colonies (undifferentiated ES/iPS colonies) vs. colorless colonies (differentiated or not fully reprogrammed colonies) using a light microscope.

The stained plate can be stored at 4°C for up to one week.

B. Protocol for Immunocytochemistry

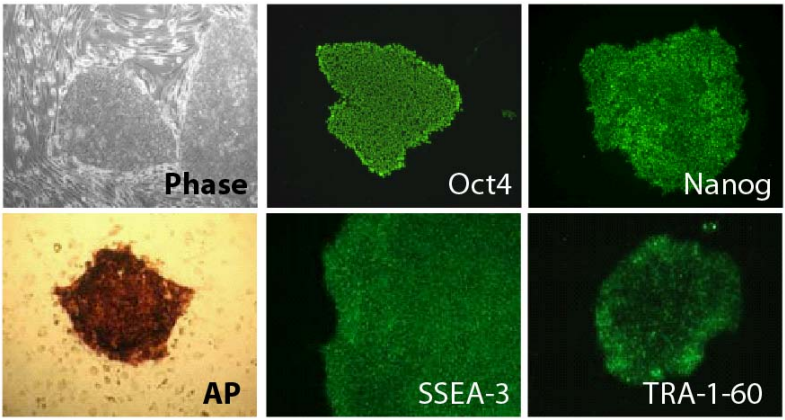
Blocking Buffer: 10% serum from the same species as secondary antibody, 0.1% Triton X-100 in PBS

Antibody Dilution Buffer: 3% serum from the same species as secondary antibody, 0.1% Triton X-100 in PBS

1. Aspirate medium from the cells that are ready for immunocytochemistry in 24-well plate.
2. Wash each well twice with 0.5 mL PBS at room temperature.
3. Fix cells with 0.5 mL 4% paraformaldehyde in PBS and incubate for 20 min at room temperature.
4. Aspirate paraformaldehyde solution and wash each well three times with PBS at 10 min intervals.

5. Add 100 µL blocking buffer per well and incubate at room temperature for 1 hour.
6. Aspirate blocking buffer and wash once with PBS.
7. Dilute primary antibody at 1:100 with antibody dilution buffer and add 100 µL diluted antibody solution to each well.
8. Incubate at 4 °C overnight.
9. The next day, aspirate antibody solution and wash each well 5 times at 10 min intervals with PBST (PBS with 0.1% Triton X-100) and PBS alternatively.
10. Dilute fluorophore-conjugated secondary antibody in antibody dilution buffer according to manufacturer's suggestions. (Use fluorophore-conjugated anti-rabbit IgG for Nanog and Oct4; use fluorophore-conjugated anti-mouse IgM secondary antibody for TRA-60-1 and SSEA3.)
11. Add 100 µL diluted secondary antibody solution to each well and incubate at room temperature for 1 hour. Protect the plate from light.
12. Aspirate secondary antibody solution and wash each well 4 times at 10 min intervals with PBST and PBS alternatively. Protect the plate from light.
13. (Optional) For nuclear visualization, cells can be incubated with 2 µg/mL DAPI solution at room temperature for 10 min, followed by 5 times PBS wash at 5 min intervals. Protect the plate from light.
14. Cells are ready for visualization under fluorescence or confocal microscope.

C. Sample results



Human induced pluripotent stem cells (hiPSCs) stained with transcription factor Nanog and Oct4 antibodies, and cell surface antigen TRA-1-60 and SSEA3 antibodies, and Alkaline Phosphotase Staining Kit. For Nanog and Oct4, the secondary antibody used was Alexa Fluor 488 (green) anti-rabbit IgG. For TRA-1-60 and SSEA3, the secondary antibody used was Alexa Fluor 488 (green) anti-mouse IgM secondary antibody. The image of AP staining was taken under phase contrast microscope. Other images were taken with florescence microscope.

III. References

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S. (2007) Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 131, 861-872.

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Slukvin I.I., Thomson J.A. (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917-1920.

IV. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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V. Licensing and Warranty

Use of the SBI Human ES/iPS Cell Characterization Kit (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended,

or warranted for use in humans or for therapeutic or diagnostic use.

Limited Warranty

SBI warrants that the Product meets the specifications described in the accompanying Product Analysis Certificate. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

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