Applied StemCell, Inc.
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Datasheet

Human ES/iPS Cell Characterization Kit

Product Information

Catalog Number **ASK-3006**

Size 20 reactions

Description ASC's Human Embryonic Stem/ Induced Pluripotent Stem (hES/iPS) Cell Characterization Kit is designed for users to examine the pluripotent status of hES/iPS cell by analyzing marker protein expression. With a standard cell culture and immunofluorescence staining protocol, our optimized, ready-to-use kit provides a fast, convenient, sensitive method for detecting the pluripotent status of hES/iPS cells. Five commonly used marker antibodies, anti-OCT4, anti-SOX2, anti-SSEA-4, anti-TRA-1-60 and anti-TRA-1-81, are included in the kit with optimized concentration for immunofluorescence staining. OCT4 and SOX2 are transcription factors highly expressed in undifferentiated ES and embryonic germ (EG) cells [1,2]. SSEA-4 is a globoseries carbohydrate antigen present on the surface of human ESC [3,4]. TRA-1-60 and TRA-1-81 antigens are expressed on the surface of human teratocarcinoma stem cells (EC), hEGC and hESC [5]. In addition, ASC's hES/iPS Cell Characterization Kit provides a rapid and sensitive alkaline phosphatase (AP) activity test. AP is a stem cell membrane marker and elevated expression of AP is associated with the pluripotent status [6].

Application Immunocytochemistry or Immunohistochemistry. Specific for hES/iPS cells.

Storage and Handling All regents supplied here are sufficient for 20 reactions according to the protocol below and also available from Applied StemCell Inc. separately.

Material	Amount	Conc.	Catalog No.	Storage & Stability
Anti-OCT4 (h)	800 µL	1x	ASA-0110	Store at +4˚C for short term (up to 6 weeks). For long term storage, aliquot and store at -20˚C to -80˚C. Avoid repeat freeze/thaw cycles.
Anti-SOX2 (h)	800 µL	1x	ASA-0120	
Anti-SSEA-4	800 µL	1x	ASA-0150	
Anti-TRA-1-60	800 µL	1x	ASA-0160	
Anti-TRA-1-81	800 µL	1x	ASA-0170	
Fixation solution*	15 mL	1x	ASB-0101	
Goat-anti-mouse *	3 mL	1x	ASA-0001 **	4˚C/-20˚C (1 year); Avoid repeat freeze/thaw cycles.
Goat-anti-rabbit *	2 mL	1x	ASA-0003 **	
DNA staining solution *	5 mL	1x	ASB-0104	
Perm solution	15 mL	1x	ASB-0102	4˚C/-20˚C (1 year)
Blocking solution	15 mL	1x	ASB-0103	4˚C/-20˚C (1 year)
Mounting solution *	4 mL	1x	ASB-0105	4˚C/-20˚C (1 year)
AP test solution *	1.5 mL	1x	ASB-0001	4˚C/-20˚C (1 year)

* Light sensitive, keep in dark.

** ASA-0001 and ASA-0003 is superior red-fluorescent Alexa Fluor 594 dye based 2nd antibody which gives off a red fluorescence with wavelength at 617 nm. But we also provide other color 2nd antibody according to customer's requirement.

Shipping Dry Ice

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Test Conditions	Cells are grown and passed to determine sterility and mycoplasma for all lots. In addition, viability is determined prior to and after freezing. All cells are confirmed to be highly pure by molecular, cellular, or protein assays with their specific markers.
Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Materials and Instruments Required but Not Provided

Required	Recommended
Phosphate buffered saline (PBS)	ThermoFisher Scientific, catalog No.12-565-88
Microscope cover slip	ThermoFisher Scientific, catalog No. 12-550-34
Microscope slides	Electron Microscopy Sciences catalog No. 72991- 4S
Stainless steel forceps	The Lab Depot, Inc. catalog No. 52858-000
Parafilm	
Fluorescence microscope or confocal microscope	
4˚C incubator	

Protocol

1. Standard cell culture protocol is recommended. Let cells grow on 13 mm cover slips.
2. Fix cells by immersing the cover slips in Fix solution at room temperature for 1-2 hours.
3. Wash cells 3 times each for 5 minutes with PBS at room temperature. For AP staining, skip to 18.
4. Permeabilize cells by immersing the cover slips in Perm solution for 30 minutes at room temperature.
5. Wash cells 2 times with PBS at room temperature.
6. Block cells in Block solution for 1 hour at room temperature.
7. Wash cells 3 times each for 5 minutes with PBS at room temperature.
8. Pipette 30 µL 1st antibody solution on the parafilm slide, put one cell cover slip on it by forceps, make sure the cell side face downward to immerse cells in antibody solution. Place the parafilm in a humidified chamber in 4˚C incubator, incubate overnight.
9. Alternatively, leave the humidified chamber from step #8 at room temperature for 2 hours.
10. Reverse the cell slices, let the cell side face upward. Aspirate the 1st antibody solution. Wash cells 3 times each for 5 minutes with PBS at room temperature.
11. Aspirate PBS. Add 1 drop (~45 µL) 2nd antibody solution to each slide according Table 1 (step #12). Prevent sample from light by a dark cover. Incubate for 1 hour at room temperature.
12. Table 1. Secondary antibody selection

1st antibody	OCT4	SOX2	SSEA-4	TRA-1-60	TRA-1-81
2nd antibody	goat-anti-rabbit	goat-anti-rabbit	goat-anti-mouse	goat-anti-mouse	goat-anti-mouse

13. Aspirate the 2nd antibody solution and wash cells 2 times by PBS each for 5 minutes in dark at room temperature.
14. Aspirate PBS. Add 1 drop (~45 µL) DNA staining solution on cell slide. Incubate for 8 minutes in dark at room temperature.
15. Aspirate DNA staining solution and wash cell cover slips by PBS in dark at room temperature.
16. Label glass slides at first. Place one drop (~20 µL) mounting solution to labeled glass slide. Put the corresponding cell cover slip on the drop, make sure the cell side face downward. Put a bigger glass cover slip on it. Package glass slides by aluminum foil and store them up to 7 days in 4˚C till reading or taking pictures under fluorescence microscope or confocal microscope.
17. Move glass slides under fluorescence microscope or confocal microscope, adjust the excitation wavelength according to 2nd antibodies, read or take pictures.
18. For AP staining, add 50 µL AP test solution to cell cover slip, staining 0.5-2 hours at room temperature. Read or take pictures under microscope.

Result Analysis and Data Attachment

All pictures attached here are obtained by common fluorescence microscope. For best quality of pictures, confocal microscope is strongly recommended. Positive signal of anti-Sox2, anti-Oct4, anti-SSEA-3, anti-TRA-1-60 and anti-TRA-1-81 should be colored red in undifferentiated human ES/iPS cells, but undetectable in feeder or any differentiated human ES/iPS cells. DNA staining should be blue in all cells. AP activity test should be navy in undifferentiated human ES/iPS cells but stainless or pale lilac in feeder or any differentiated human ES/iPS cells.

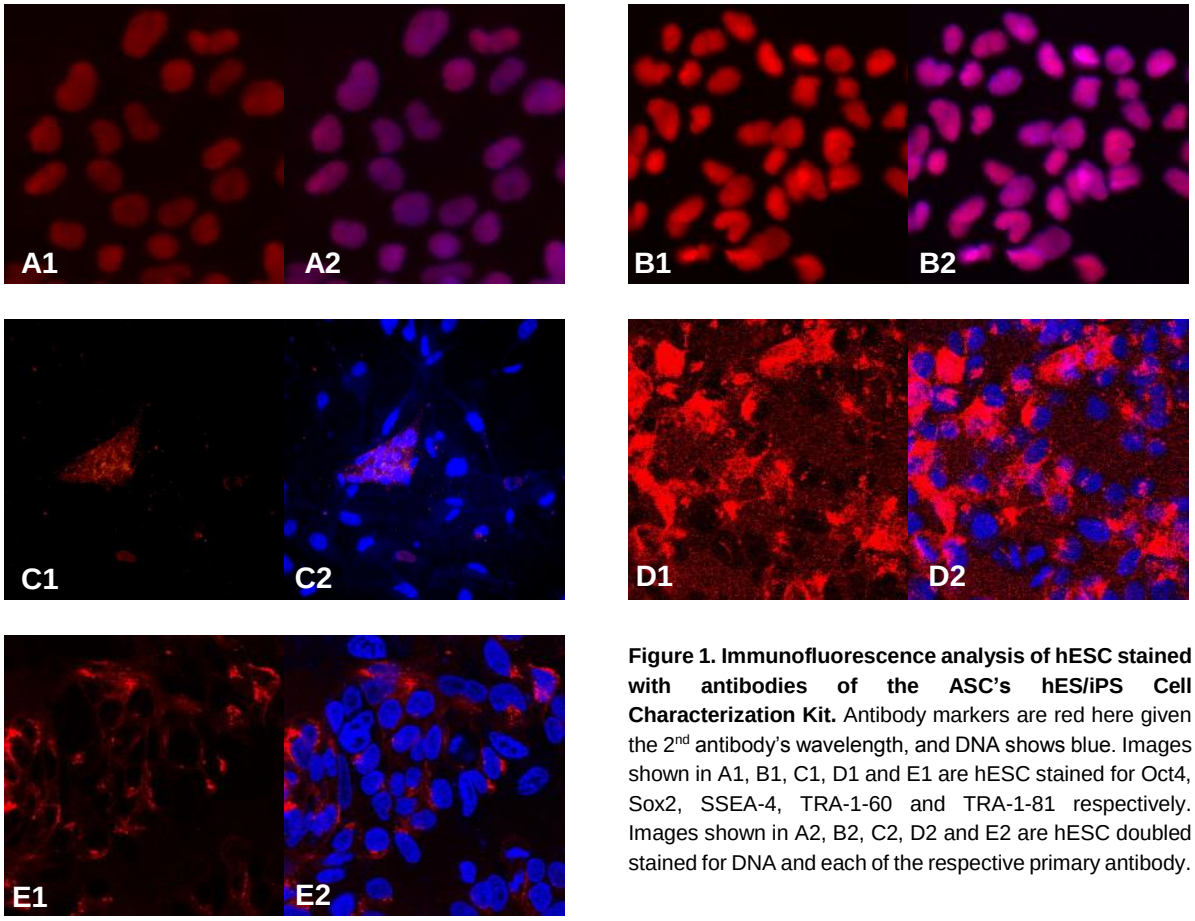

Figure 1. Immunofluorescence analysis of hESC stained with antibodies of the ASC's hES/iPS Cell Characterization Kit. Antibody markers are red here given the 2nd antibody's wavelength, and DNA shows blue. Images shown in A1, B1, C1, D1 and E1 are hESC stained for Oct4, Sox2, SSEA-4, TRA-1-60 and TRA-1-81 respectively. Images shown in A2, B2, C2, D2 and E2 are hESC doubled stained for DNA and each of the respective primary antibody.

References

1. Looijenga LH, Stoop H, de Leeuw HP, de Gouveia Brazao CA, Gillis AJ, van Roozendaal KE, van Zoelen EJ, Weber RF, Wolffenbuttel KP, van Dekken H, Honecker F, Bokemeyer C, Perlman EJ, Schneider DT, Kononen J, Sauter G, Oosterhuis JW (2003). "POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors". Cancer Res. 63: 2244–2250.
2. 2. Zhao R, Daley GQ (2008). "From fibroblasts to iPS cells: induced pluripotency by defined factors". J. Cell. Biochem. 105: 949–955.
3. 3. Masui, Shinji (2007). "Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells". Nature Cell Biology 9: 625–635.
4. 4. Przyborski SA (2001). "Isolation of human embryonal carcinoma stem cells by immunomagnetic sorting". Stem Cells 19: 500-504.
5. 5. Solter D, Knowles BB(1978). "Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1)". Proc Natl Acad Sci USA 75: 5565–5569.
6. 6. Andrews PW, Banting GS, Damjanov I, Arnaud D, Avner P(1984)." Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells". Hybridoma 3: 347-361.
7. 7. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM.(1998) "Embryonic stem cell lines derived from human blastocysts". Science 282:1145-1147