Pluripotency markers staining

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ABSTRACT

This protocol describes the standard procedure for staining pluripotency markers, e.g. OCT4, SSEA4, alkaline phosphatase, and etc. on human pluripotent stem cells (hPSCs).

Protocol overview

A. Immunofluorescence staining
B. Alkaline phosphatase staining

General notes

Throughout this protocol, the term hPSC is used to collectively refer to both hiPSCs and hESCs. All described procedures have been tested and work equally well for hiPSCs and hESCs.

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MATERIALS TEXT

Item	Vendor	Catalog #
Para formaldehyde, PFA	Sigma	Millipore Sigma 158127
Bovine Serum Albumin (BSA)	Sigma	A4503
OCT4 primary antibody	DSHB	PCRP-POU5F1-1D2
SSEA4 primary antibody	DSHB	MC-813-70 (SSEA-4)
Donkey anti Mouse IgG (H+L) Highly Cross Adsorbed Secondary Antibody, Alexa Fluor 594	Thermo Fisher	A21203
NBT/BCIP substrate	Sigma/Roche	11681451001
NBT/BCIP substrate	Vector lab	SK-5400
Triton-X100	Sigma	X100
DAPI	Sigma	D9542

A. Immunofluorescence staining 1h 50m

1 Wash cells once with PBS

Phosphate buffer saline, PBS, pH 7.4

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1.1

A	B
NaCl	137 mM
KCl	2.7 mM
Na2HPO4	10 mM
KH2PO4	1.8 mM
CaCl2•2H2O	1 mM
MgCl2•6H2O	0.5 mM

15m

2 Fix cells with 4% PFA at **Room temperature** for **00:15:00**

You should not fix cells if staining for cell surface proteins

2.1 **4% PFA, pH 7.4**

A	B
PBS	500 ml
PFA	20 g
NaOH	Adjust pH to 7.4

Final volume: 500 ml

5m

3 Wash cells twice with PBS, incubate **00:05:00** in between washes at **Room temperature**

30m

4 Incubate in PBST for **00:30:00** at **Room temperature** to permeabilize cell membrane

4.1 **PBST, pH 7.4**

A	B
PBS	1x
Triton-X100	0.3%

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5 Incubate in 3% Bovine Serum Albumin (BSA) for **01:00:00** at **Room temperature** 1h

5.1 **3% Bovine Serum Albumin (BSA)**

A	B
PBS	500 ml
BSA	15 g

6 Incubate with primary antibody (1:200) in 3% BSA at **4 °C** **Overnight** 1h

7 Wash three times with PBS, incubate **00:05:00** in between washes at **Room temperature** 5m

8 Incubate with secondary antibody (1:1,000) in 3% BSA at **Room temperature** for **01:00:00** in the dark and in a humidified chamber. 1h

9 Wash once with PBS

10 Incubate with 0.1 µg/ml DAPI for **00:05:00** at **Room temperature** 5m

11 Wash twice with PBS, incubate **00:05:00** in between washes at **Room temperature** 5m

12 Image cells, seal the plate with parafilm, and wrap with foil for longer storage at **4 °C** . Florescence is usually stable for several weeks.

B. Alkaline phosphatase staining 20m

Wash once with PBS

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13

14 Fix cells with cold 4% PFA, **00:10:00** at **Room temperature** . 10m

15 Wash twice with PBS

16 Incubate cells with 100 mM Tris, pH 9.5, **00:10:00** at **Room temperature** 10m

16.1 **100 mM Tris, pH 9.5**

A	B
Tris	6.57g
HCl	Adjust pH to 9.5

Final volume: 500 ml

17 Add NBT/BCIP substrate in 100 mM Tris, and let reaction develop at **Room temperature** , avoiding light until color becomes clearly apparent in control cells.

We have used NBT/BCIP substrate from two different vendors, which can be found in the materials list. The formulation for each are as follows:

A	B	C
NBT/BCIP substrate	Vector	1 drop of each reagent in 6 ml
NBT/BCIP substrate	Roche	100 µl to 10 ml

18 Wash in PBS and keep in PBS (violet product is soluble in organic solvent).

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