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FRONTIERS IN LIFE SCIENCE

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iPSelector <Anti-LNFP I, Human, Mouse-Mono(R-17F)>

Research use only. Not for diagnostic purpose.

This antibody has been commercialized under a license from Ritsumeikan University.

Description

Product Name: iPSelector <Anti-LNFP I, Human, Mouse-Mono(R-17F)>

Catalog Number: FDV-0014A, FDV-0014B, FDV-0014P

Volume: 25 μL (FDV-0014A), 100 μL (FDV-0014B), 10 μL (FDV-0014P)

Lot Number: see vial label

Host Species and Clonality: Mouse Monoclonal

Clone: R-17F

Specificity: This antibody recognizes lacto-*N*-fucopentaose I (LNFP I: Fucα1–2Galβ1–3GlcNAcβ1–3Galβ1–4Glc) on a glycolipid / glycoprotein. R-17F epitopes are expressed on undifferentiated human induced pluripotent stem (iPS) / embryonic stem (ES) cells but not on human embryonal carcinoma (EC) cells nor on differentiated human iPS/ES cells.

Isotype and Subclass: IgG1

Formulation: Phosphate Buffered Saline (PBS) containing 50% Glycerol, contains no preservative.

Purification: Protein A Purified

Concentration: 1.0 mg/ml

Verified Species Reactivity: Human --- Other species not tested.

Immunogen: Human iPS cell line, Tic, derived from human fetus lung cells (MRC-5).

Applications: Western Blot (1:2,000), Immunocytochemistry, Flow Cytometry, Functional Application

(Optimal working dilutions should be determined experimentally by each laboratory for each application.)

Storage: -20°C (Avoid repeated freeze-thaw cycles.)

Product Background

Clone SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81 antibodies are well-known as human iPS/ES cell-marker antibodies. Since SSEA-3 antibody was originally generated against mouse embryo and the other antibodies were against human EC cells, these antibodies recognize not only human iPS/ES cells but also human EC cells.

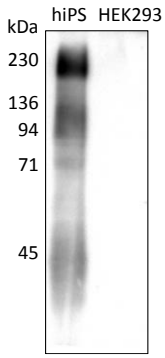
Table1. Binding Activity of Antibodies to Cells

	R-17F	TRA-1-60	TRA-1-81	SSEA-3	SSEA-4
Tic (iPS)	++++	++++	++++	++++	++++
KhES-3 (ES)	+++	++++	++++	+++	++++
H9 (ES)	++++	++++	++++	+++	++++
2102Ep (EC)	+/-	++++	++++	+++	+++

R-17F is a novel mouse monoclonal antibody generated by using a human iPS cell line as an immunogen. It is specific to human iPS/ES cells and does not essentially cross-react against human EC cells (Table 1, ref. 1). This **R-17F** antibody also stains entire surface of human iPS/ES cell membranes evenly, while the staining by SSEA-3 and SSEA-4 antibodies are not uniformly (ref. 2). In addition, **R-17F** is reported to exhibit potent dose-dependent cytotoxicity when added to living human iPS/ES cells (ref. 2 & 3).
R-17F is a beneficial tool for the selective detection, staining and removal of undifferentiated of human iPS/ES cells in regenerative medicine.

Application Data

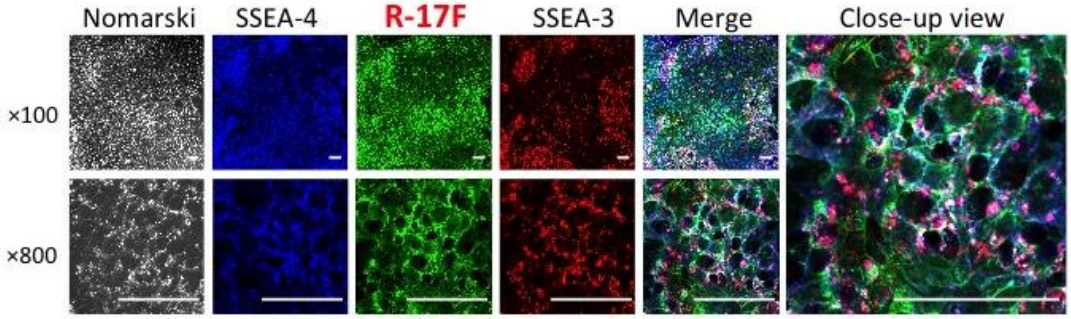
Western Blotting



Sample: 5 μg cell lysate in each lane.
Left: human iPS cells (LNFP I positive)
Right: HEK293 (Negative Control)
Dilution: 1:2,000
Secondary antibody: Anti-Mouse IgG, Goat-Poly, HRP (Kirkegaard & Perry Laboratories, #5220-0337)
Chemiluminescence Substrate: Trident plus Western HRP Substrate (GeneTex International Corporation, #GTX400006)
Detection: LuminoGraph I (ATTO) with 1min exposure

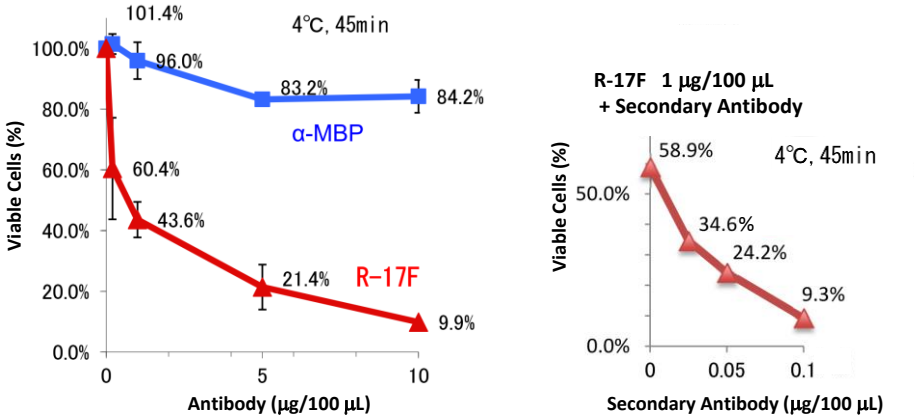
One major positive band and several minor bands were specific to human iPS cells, and any positive band was not obtained with HEK293 cells.

Immunocytochemical Staining



Cultured human iPS cells were stained with R-17F, SSEA-3, and SSEA-4 antibodies. [bars: 100 μm]
R-17F stained the entire surface of the cell membranes equally, while the staining by SSEA-3 and SSEA-4 antibodies are not evenly. This suggests that R-17F epitope is expressed ubiquitously all over the human iPS cells.

Functional Application (Cytotoxic effects on undifferentiated iPS/ES cells)



R-17F is reported to exhibit potent dose-dependent cytotoxicity when it is added to living human iPS/ES cells.

[Left] After the incubation of iPS cell suspension with R-17F at 4°C for only 45 minutes, the percentage of viable cells decreased concentration-dependently (red triangles).
Blue squares: effects of the isotype (IgG1)-matching control antibody (anti-α-MBP) as Negative Control

[Right] When R-17F-treated iPS cells were incubated with a small amount (0.025-0.1 μg) of the secondary antibody (goat anti-mouse IgG1 antibody), the cytotoxic effect of R-17F was enhanced significantly in a dose-dependent manner (red triangles).

Reference

1. Kawabe, K., *et al*., *Glycobiology*, **23**, 322 (2013), doi: 10.1093/glycob/cws159
2. Matsumoto, S., *et al*., *J. Biol. Chem.*, **290**, 20071 (2015), doi: 10.1074/jbc.M115.657692
3. Nakao, H., *et al.*, *Glycoconj. J.*, **34**, 779 (2017), doi: 10.1007/s10719-0169-9710-2

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