funakoshi
FRONTIERS IN LIFE SCIENCE

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

Catalog NO. FDV-0014B

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.

Our **iPSelector (clone 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 iPSelector (clone 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, iPSelector (clone R-17F) is reported to exhibit potent dose-dependent cytotoxicity when added to living human iPS/ES cells (ref. 2 & 3). iPSelector (clone R-17F) is a beneficial tool for the selective detection, staining and removal of undifferentiated of human iPS/ES cells in regenerative medicine.

Table1. Binding Activity of Antibodies to Cells

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

Description

Catalog Number: FDV-0014B

Size: 100 μL

Lot No.: see vial label

Host Species and Clonality: Mouse Monoclonal

Clone name: 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 * Note: Other species not tested.

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

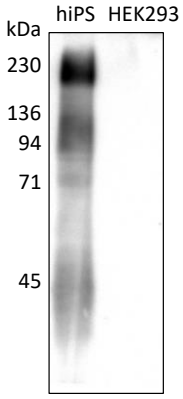
Storage: -20°C (Avoid repeated freeze-thaw cycles.)

Application and Recommended usage

- Western blotting	1/2,000
- Immunohistochemistry	Optimal dilutions should be empirically determined for each experiments
- Flow cytometry	Optimal dilutions should be empirically determined for each experiments
- Functional applications	Optimal conditions should be empirically determined for each experiments

Reference and Application Data

Western Blotting

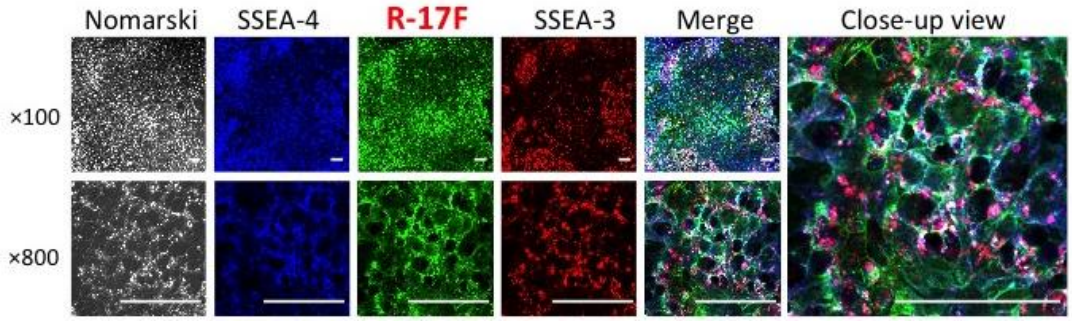


Sample: 5 μg cell lysate in each lane.
Left: human iPS cells (LNFP I positive)
Right: HEK293 (Negative Control)
Dilution: 1:2,000
Secondary antibody: Anti-Mouse IgG, Goat-Poly, HRP
(Kirkegaard & Perry Laboratories, #5220-0337)
Chemiluminescence Substrate: Trident plus Western HRP Substrate
(GeneTex,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

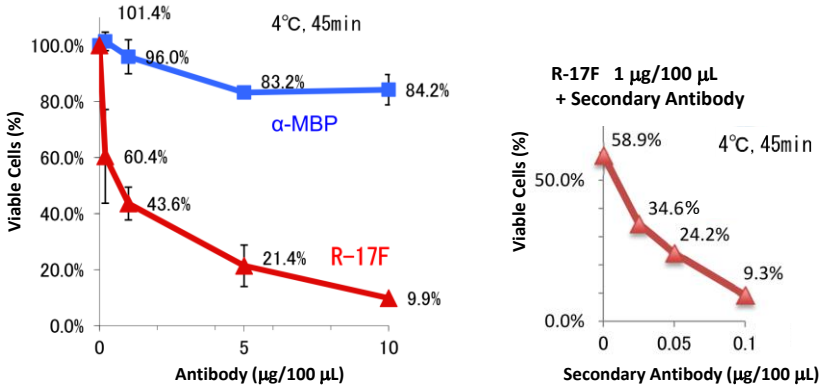
Immunocytochemical Staining

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, *et al.*, *Glycobiology*, **23**, 322-336 (2013) A novel antibody for human induced pluripotent stem cells and embryonic stem cells recognizes a type of keratan sulfate lacking oversulfated structures
2. Matsumoto, *et al., J. Biol. Chem.*, **290**, 2007-200851 (2015) A Cytotoxic Antibody Recognizing Lacto-N-fucopentaose I (LNFP I) on Human Induced Pluripotent Stem (hiPS) Cells
3. Nakao, *et al.*, *Glycoconj. J.*, **34**, 779-787 (2017) Characterization of glycoproteins expressing the blood group H type 1 epitope on human induced pluripotent stem (hiPS) cells

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