Collaborative development product with National Institute of Advanced Industrial Science and Technology.

Wako innovación tecnológica para laboratorio Rafer

Life Science

Capable of monitoring the increase and decrease of undifferentiated cells

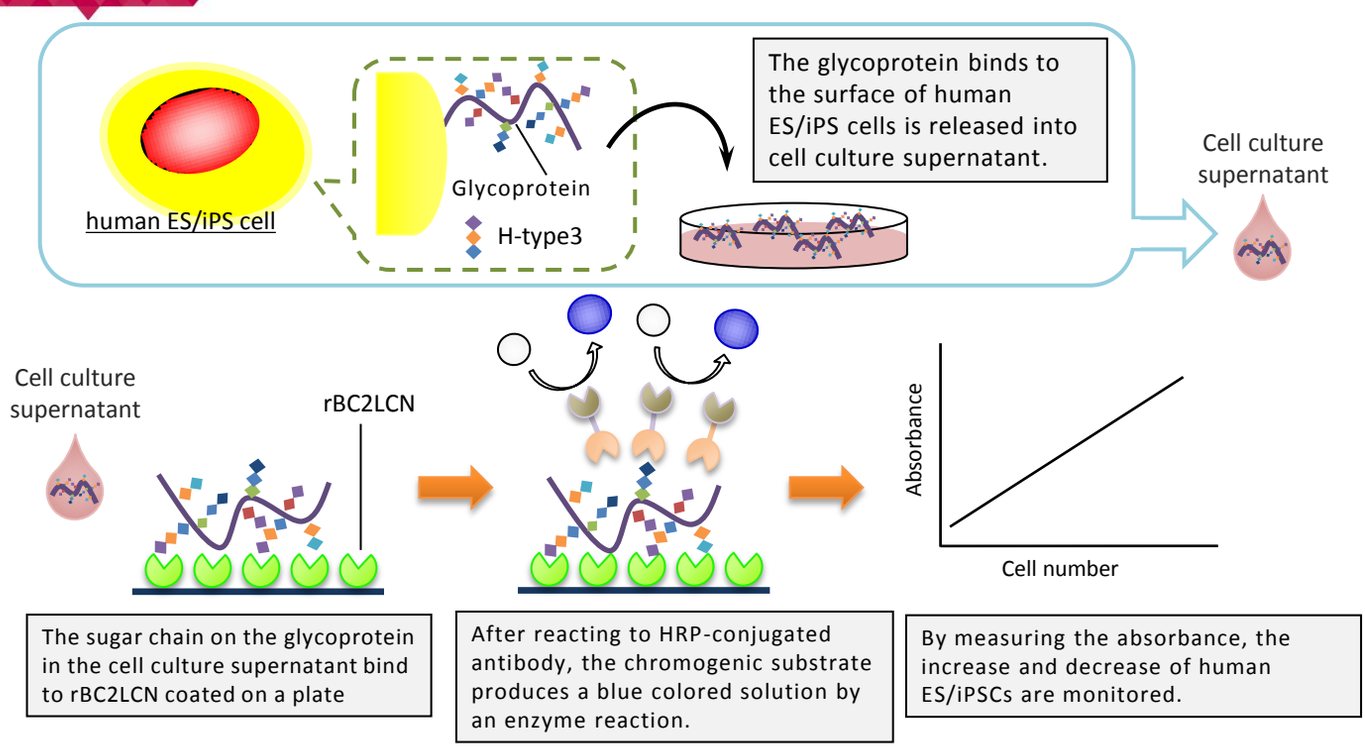
Human ES/iPS Cell Monitoring Kit

rBC2LCN lectin exhibits significant affinity to a mucin-type *O*-glycan sugar chain called H-type3 (Fuc α1-2Gal β1-3GalNAc) displayed on the podocalyxin binds to the surface of human pluripotent stem cells (hPSCs), human ES cells and human iPS cells. Therefore rBC2LCN is reported as a marker of hPSCs.
The glycoprotein recognized by rBC2LCN is released from hPSCs into cell culture supernatant. This kit can quantitatively determine the released glycoprotein by rBC2LCN-antibody sandwich assay and estimate the number of hPSCs. Because, cell culture supernatants, but not cells itself, are used as sample targeted for assay, it is easily capable of monitoring the increase and decrease of hPSCs in a noninvasive manner during continuous cell culture.

Features

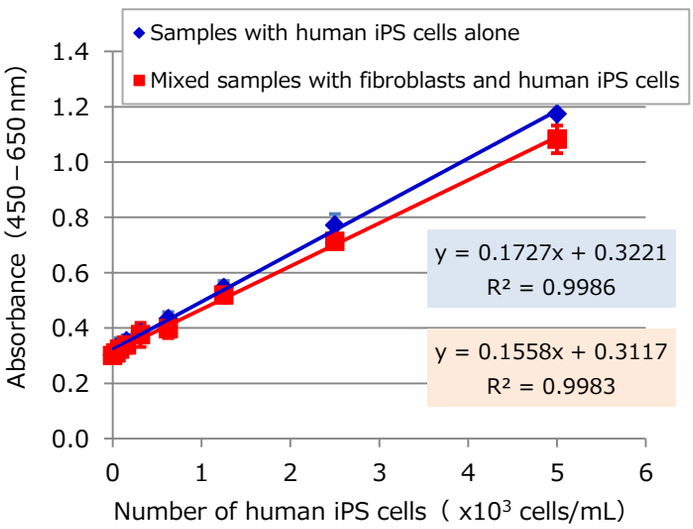
- Increase or decrease in the number of undifferentiated cells can be monitored by analyzing culture the supernatants of undifferentiated cells that are undergoing differentiation.
- Culture supernatants (50 µL) are used for the assay. Cells are not processed for the analysis and can remain in culture.
- As an ELISA-based method is used, multiple samples can be processed using a simple procedure.

Principle



Product Name	Pkg. Size	Wako Cat. No.	Grade	Storage Condition
Human ES/iPS Cell Monitoring Kit	96 tests	299-78301	for Regenerative Medical Research	Keep at 2~10℃

Data 1: Spiking experiment using hiPS cell culture supernatants mixed with culture supernatants of differentiated cells



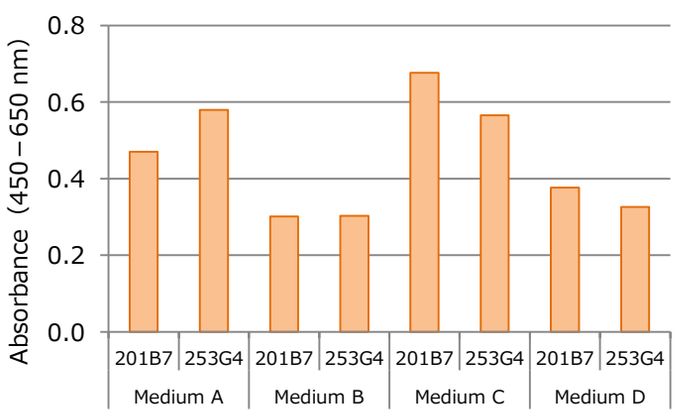
The human iPS cell line 201B7 was cultured in StemSure™ hPSC media Δ. Culture supernatants were sampled on the day following the date of medium replacement. Cell counts were taken at the time of sampling. The culture supernatants were serially diluted with StemSure hPSC media Δ and analyzed using this kit.
A regression equation with a high correlation coefficient was obtained from the obtained results. Based on the regression equation, the lower limit of detection* for human iPS cells under this culture condition was calculated as 23 cells/mL.

In addition, mixed samples were analyzed to determine whether human iPS cell culture supernatants can be assayed in the presence of fibroblast cell culture supernatant containing serum. Mixed culture supernatants were prepared by adding human iPS cell culture supernatants to fibroblast culture supernatants such that the ratio of iPS cells was 0 – 20% of total cell number (0 - 2 x 10^4 cells/mL; total cell number 1 x 10^5 cells/mL). Assays were performed using this kit. A regression equation with a high correlation coefficient was obtained from the results. Thus, human iPS cell culture supernatants could be analyzed even when they were prepared in the presence of serum and fibroblasts. Based on the regression equation obtained with the mixed samples with fibroblasts and human iPS cells, the detection limit for human iPS cells under this condition was calculated as 170 cells/mL.

Similarly, assays were successfully performed in the presence of culture supernatants of other differentiated cells, including human articular chondrocytes and HeLa cells.

*Lower limit of detection : Number of cells calculated as the mean average plus 3.3-fold the standard deviation of the measure of the negative control media.

Data 2: Comparison of different culture media



After culturing human iPS cells: iPS cell line 201B7, 253G4 in the Medium A, B, C and D under undifferentiated condition, the culture supernatants were harvested on the day following the date of medium replacement. The cell number of each condition was counted, and the culture supernatants was diluted with individual culture medium to 2,000 cells/mL. The signal intensity of different culture supernatant was measured with this kit. The data indicate that the correlation between signal intensity and cell number is substantially affected by the media.

NOTE:

- The relation between signal intensity and cell number (cells/mL) is greatly affected by culture conditions such as cell strains and culture medium. Prepare standard curve for each cell strain and undifferentitated maintenance culture condition, respectively.
- The levels of signal intensity obtained under different culture conditions cannot be compared to evaluate undifferentiated cells. Identical culture conditions should be used.

Reference

Tateno, H., Onuma, Y., Ito, Y., Hiemori, K., Aiki, Y., Shimizu, M., Higuchi, K., Fukuda, M., Warashina, M., Honda, S., Asashima, M. and Hirabayashi, J.:*Sci. Rep.*, **4**, 4069 (2014).

innovación tecnológica para laboratorio Rafer

www.rafer.es

Barcelona 93 645 50 28 barcelona@rafer.es
Bilbao 94 499 85 80 bilbao@rafer.es
La Coruña 981 93 89 26 galicia@rafer.es
Madrid 91 365 15 70 madrid@rafer.es
Málaga 639 359 792 malaga@rafer.es
Sevilla 954 369 334 sevilla@rafer.es
Valencia 96 340 48 00 levante@rafer.es
Zaragoza 976 23 74 00 rafer@rafer.es
Lisboa 21 154 19 98 lisboa@rafer.es