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Code No. 187-03501 (100 μL)

rBC2LCN-635 (AiLecS1-635), AF

rBC2LCN is a recombinant protein of N-terminal domain of BC2L-C and has been reported as a marker of undifferentiated human pluripotent stem cells (hPSCs), human ES cells and human iPS cells. This product is the rBC2LCN labeled with red fluorescence dye, spectral similar to Cy5 and Alexa Fluor® 647. rBC2LCN specifically recognize a sugar chain which exists on the surface of hPSCs. When you add rBC2LCN-635 to the medium which is used for hPSCs culture, you can stain the living hPSCs. rBC2LCN-635 is not toxic to cells, therefore you can culture hPSCs without any effect on the growth and pluripotency in the medium containing rBC2LCN-635. Also, this product can be used for flow cytometry.
This product is produced using non-animal ingredients as raw materials.
This product is for laboratory research use only; use in any such application is the responsibility of the user.

【Formulation】 : Phosphate Buffered Saline sterilized with 0.1 μm filter

【Working Dilution】 : Live Cell Imaging 1 : 100 ～ 1 : 1,000
Flow Cytometry 1 : 100 ～ 1 : 1,000

【Protocol for Live Cell Imaging】
1) Culture hPSCs in appropriate culture conditions.
2) Add 1 ～ 10 μL of rBC2LCN-635 per medium 1 mL.
3) Incubate the cells over 30 min under appropriate conditions.
4) Replace the medium containing rBC2LCN-635 to a fresh medium, HBSS(+) or D-PBS(-).
5) View and analyze the cells on an imaging instrument (excitation 634 nm, emission 654 nm).
※ Step 4 may be omitted.

【Protocol for Flow Cytometry】
1) Dissociate the hPSCs and wash with FCM* buffer once. Centrifuge and discard the supernatant.
2) Resuspend the cells with FCM* buffer to prepare cell suspension of approximately 5×10^6 cells/mL.
3) Add 1 ～ 10 μL of rBC2LCN-635 per 1 mL of cell suspension.
4) Incubate the cells in the dark over 30 min at room temperature.
5) Centrifuge the cell suspension at 1,000 rpm for 3 min, and discard the supernatant.
6) Wash the cells with 1 mL of FCM* buffer, centrifuge the cell suspension at 1,000 rpm for 3 min and discard the supernatant.

7) Suspend the washed cells in appropriate volume of FCM* buffer.
8) Analyze the cells by flow cytometry.
＊FCM buffer : D-PBS(-) containing 10 mmol/L EDTA and 1% FBS, and so on.

【Attention】
1) Staining with the rBC2LCN-635 persists for a few days even after removing the rBC2LCN-635.
2) For continuous observations, it might be better to add the same concentration of rBC2LCN-635 at the daily medium change. Cytotoxicity of the rBC2LCN-635 has not been observed.
3) rBC2LCN-635 is applicable to not only live cell imaging, but also staining fixed cells.
4) The signal of background might become higher when the cells are cultured with the medium containing serum.
5) When sorting cells by flow cytometry, please add Y-27632 at a final concentration of 10 μmol/L to the FCM buffer at the time of cell dispersion.

【Storage】 : Store at -20℃, in the dark
After thawing, store at 2 ～ 10℃. Avoid repeating freeze-thaw.

【References】

1) Onuma, Y., Tateno, H., Hirabayashi, J., Ito, Y. and Asashima, M.: *Biochem. Biophys. Res. Commun.*, **431**, 524, (2013).
2) Tateno, H., Matsushima, A., Hiemori, K., Onuma, Y., Ito, Y., Hasehira, K., Nishimura, K., Ohtaka, M., Takayasu, S., Nakanishi, M., Ikehara, Y., Nakanishi, M., Ohnuma, K., Chan, T., Toyoda, M., Akutsu, H., Umezawa, A., Asashima, M and Hirabayashi, J.: *Stem Cells Transl. Med.*, **2**, 265, (2013).
3) 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).

【Package】

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187-03501	100 μL

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