



◆ YZWJs524/AB II-KO-07

(YZWJ; iPS cells with the most common HLA type in Japan<sup>(\*)</sup>)

|                                      |                                                                                                                                                                                                            |                   |                 |
|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------|
| Clone ID                             | YZWJs524/AB II-KO-07                                                                                                                                                                                       | Product           | Human iPS cells |
| Source                               | Cord Blood, Human                                                                                                                                                                                          | Race              | Japanese        |
| Passage No.                          | 22                                                                                                                                                                                                         | Gender            | Male            |
| Label Name                           | Fit__13SKC(YZWJs524)-250610                                                                                                                                                                                | Manufacture Dates | 01-Jul-2025     |
| Culture medium                       | StemFit AK03N                                                                                                                                                                                              | Substrate         | iMatrix-511MG   |
| Culture Method                       | Feeder-free <sup>(*)2)</sup>                                                                                                                                                                               |                   |                 |
| Genome-editing techniques            | CRISPR-Cas9 <sup>(*)3)</sup>                                                                                                                                                                               |                   |                 |
| Use and Provision of this cell stock | Please check our web site ;<br><a href="https://www.cira-foundation.or.jp/e/provision-of-ips-cells/manufacturing-flow/">https://www.cira-foundation.or.jp/e/provision-of-ips-cells/manufacturing-flow/</a> |                   |                 |

(\*)1) Reference; Okita, *et. al.*, Nat Methods. 2011 8(5): 409-412

(\*)2) Reference; Nakagawa, *et. al.*, Sci. Rep. 2014 4: 3594

(\*)3) Reference; Huaigeng Xu, *et al.* Cell Stem Cell. 2019 Apr 4;24(4):566-578.

## Test Result

| Test                                                     | Method                                                                        | Result                                                                                                                             |
|----------------------------------------------------------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|
| <b>Sterility</b>                                         | Direct Inoculation Method                                                     | Negative                                                                                                                           |
| <b>Mycoplasma</b>                                        | PCR                                                                           | Negative                                                                                                                           |
| <b>Endotoxin</b>                                         | LAL                                                                           | < 0.01523 EU/mL                                                                                                                    |
| <b>Virology</b><br>(HBV, HCV, HIV, HTLV, ParvoB19)       | PCR                                                                           | Negative                                                                                                                           |
| <b>Morphology</b>                                        | Microscope                                                                    | Consistent with human ES cells                                                                                                     |
| <b>Thawed postnatal cells</b>                            | Cell count <sup>(*4)</sup>                                                    | 2.51 x 10 <sup>5</sup> cells (Survival rate; 82.4%)                                                                                |
| <b>STR genotyping</b>                                    | PCR                                                                           | Consistent with the donor cells                                                                                                    |
| <b>Undifferentiated markers</b>                          | Flow cytometry                                                                | TRA-1-60(+) :95.45 %<br>SSEA4(+); 99.99 %<br>TRA-2-49(+); 99.51 %<br>OCT3/4(+); 98.75 %                                            |
| <b>Gene editing confirmation</b>                         | WGS                                                                           | Detected of edits                                                                                                                  |
|                                                          | Flow cytometry <sup>(*5)</sup>                                                | HLA-A(-) ; 99.93 %<br>HLA-C(+); 90.08 %                                                                                            |
| <b>Karyotype</b>                                         | G-banding                                                                     | 49,XY,+3,+13,+14[1] <sup>(*6)</sup><br>46,XY[19]                                                                                   |
| <b>CNV<sup>(*5)</sup> (*7)</b>                           | WGS, SNP                                                                      | No de novo CNVs (>1kbp) were found in COSMIC Cancer Gene Census (ver.96) and Shibata list <sup>(*8)</sup> .                        |
| <b>SNV/Indel<sup>(*5)</sup> (*7)</b>                     | WGS                                                                           | No de-novo non-synonymous SNVs/Indels were found in COSMIC Cancer Gene Census (ver.96) and Shibata list <sup>(*8)</sup> .          |
| <b>Residual guide RNA<sup>(*5)</sup></b>                 | qPCR                                                                          | Not detected                                                                                                                       |
| <b>Residual Cas9<sup>(*5)</sup></b>                      | ELISA                                                                         | 0.875 ng/mL <sup>(*9)</sup>                                                                                                        |
| <b>Cardiac differentiation<sup>(*5)</sup></b>            | Reference: “Funakoshi et al., 2016, Sci Rep.”                                 | TnT(+) = 24.89 %                                                                                                                   |
| <b>Number of proliferating cells after thawing</b>       | Counting the number of the cells after culturing for 6 days <sup>(*4)</sup> . | 7.88 × 10 <sup>5</sup> cells (Number of seeded cells : 0.65 × 10 <sup>5</sup> cells)                                               |
| <b>Corneal epithelial differentiation<sup>(*5)</sup></b> | Flow cytometry                                                                | SSEA4 (+) CD104 (+) = 16.3 % <sup>(*10)</sup><br>Measurements (%) equivalent to those of the parent strain YZWJs524 were obtained. |

(\*4) NucleoCounter® NC200

(\*5) Reference test: these are not related to the product release.

(\*6) Not qualify as a clone according to the ISCN definition

(\*7) CNV; Copy Number Variation , SNV/Indel; Single nucleotide variants /Insertion Deletion

(\*8) The PMDA Science Board “Current Perspective on Evaluation of Tumorigenicity of Cellular- and Tissue-based Products Derived from induced Pluripotent Stem Cells (iPSCs) and iPSCs as Their Starting Materials” (Cellular-

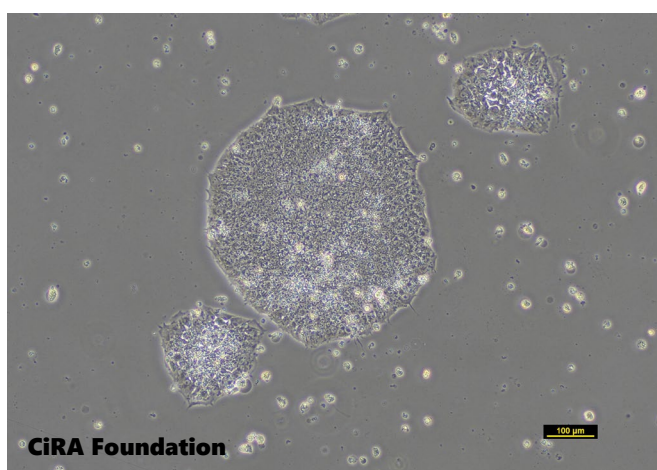
and Tissue-based Products Subcommittee, 20 August 2013)

(\*9) As a reference, the residual value of Cas9 in the cells immediately after transfection: 6~34 ng/mL

In the negative control: 1 ng/mL.

(\*10) Evaluation of PKC (Primary Knock-out Cell) as an intermediate material (test site: Department of Ophthalmology, Graduate School of Medicine, Osaka University)

## ■Image



Scale bar: 100 μm

Please contact us if you have any questions.

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