

Cell Line Name and Description: Ospedale San Raffaele (OSR) Human Induced Pluripotent Stem Cell Line
OSR_003 #56

Grade: Research-grade
Cells distributed by OSR-iPSC factory are intended for research purpose only and not for use in human.

Consent Status: They have not been consented onward for distribution or commercialization.

Starting Material Description: Human Fibroblasts derived from skin biopsy

Donor Information: female, age: 39 years old

Reprogramming Method: RNA Transfection with Oct4, Sox2, Klf4, cMyc, Nanog and Lin28

Culture System and Conditions: Basal medium: StemFlex, Substrate: rhVTN-N,
Subculture by passaging using EDTA 0.5mM. Cells cultured at 37°C, 5% CO₂.

Passage number: P14

Thaw Recommendations: 1 vial should be thawed into 1 well of a 6 well plate.
1 vial contains 1x10⁶ cells in CryoBrew frozen medium.

Test description	Method	Specification	Result
Cell Morphology	Phase contrast microscopy	- Tightly packed cells - Large nucleus to cytoplasm ratio - Homogeneous distribution	Pass
Expression of pluripotency-associated proteins	Flow cytometry	- SSEA-4 ≥ 90% - Tra-1-60 ≥ 90% - Oct4 ≥ 90% - Sox2 ≥ 90%	Pass
Differentiation	Trilineage differentiation	- Differentiation into cells of the 3 germ layers (mesoderm, endoderm and ectoderm) as shown by flow cytometry: - Endoderm: CXCR4 ≥ 90% - Ectoderm: Pax6 ≥ 90% - Mesoderm: CD140b/CD144 ≥ 90%	Pass
Mycoplasma	MycoAlert™ Mycoplasma Detection Kit	Negative	Pass
Identity	STR: profiling of 10 STR regions	- Consistent with human cell line profiling - Consistent with starting material	Pass
Karyotype	KaryoStat+ Karyotyping analysis detecting structural abnormalities of size >1-2 Mb	- Normal Karyotype, 46XX - Consistent with starting material	Pass

Compilazione ed esecuzione metodiche	Sviluppo	Approvazione
CRB - iPSC factory S. Pellegrini G. Di Giuseppe	Coordinatore iPSC factory S. Pellegrini	Responsabile CRB C. Tresoldi
Data approvazione documento: 18/12/2024		

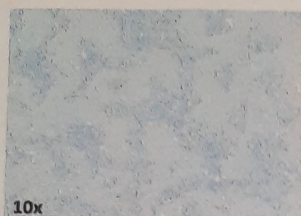


Figure 1. Morphology of iPSC OSR_003 #56 p14 on day 2 of culture in StemFlex medium. Magnification 10x.

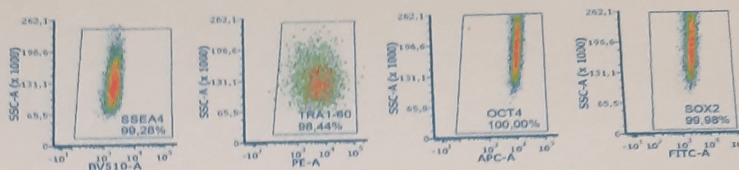


Figure 2. Dot plots of the percentage of SSEA-4, TRA 1-60, OCT4 and SOX2 expression in iPSC OSR_003 #56 p14. Gate delimitates positive events.

Gene	TH01	D21S11	D5S818	D13S317	D7S820	D16S539	CSF1PO	Amelogenin	vWA	TPOX
Fibroblasts_OS03 P3	8	30	32.2	10	12	10	12	X	16	17
iPSC_OS03 #56 p9	8	30	32.2	10	12	10	12	X	16	17

Figure 3: STR analysis for the detection of ten loci (TH01, D21S11, D5S818, D13S317, D7S820, D16S539, CSF1PO, Amelogenin, vWA, TPOX) in the fibroblast cell line (Fibroblasts_OS03 P3) and the iPSC line (OSR_003 #56 P9). The comparison of STR between the samples confirm matching genetic profiles and verify the cell identity.

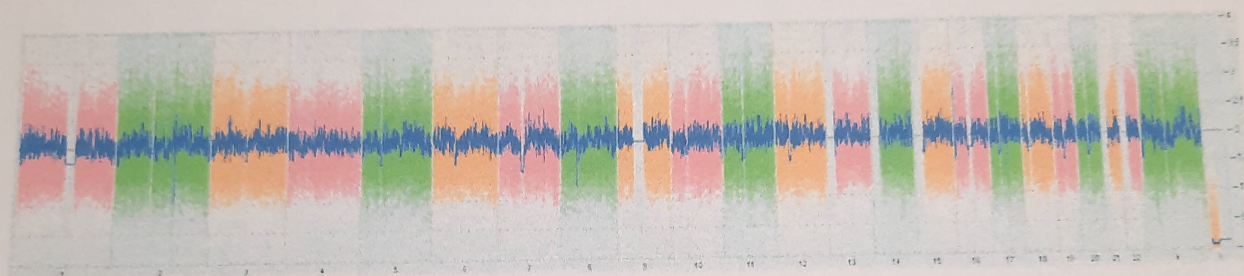


Figure 4: Whole genome view from Karyotype analysis of iPSC OSR_003 #56 p10. The whole genome view displays all somatic and sex chromosomes in one frame with high level copy number. The smooth signal plot (right y-axis) is the smoothing of the log2 ratios which depict the signal intensities of probes on the microarray. A value of 2 represents a normal copy number state, a value of 3 a chromosomal gain and a value of 1 a chromosomal loss. The pink, green and yellow colors indicate the raw signal for each individual chromosome probe, while the blue line represents the normalized probe signal used to identify copy number and aberrations (if any).

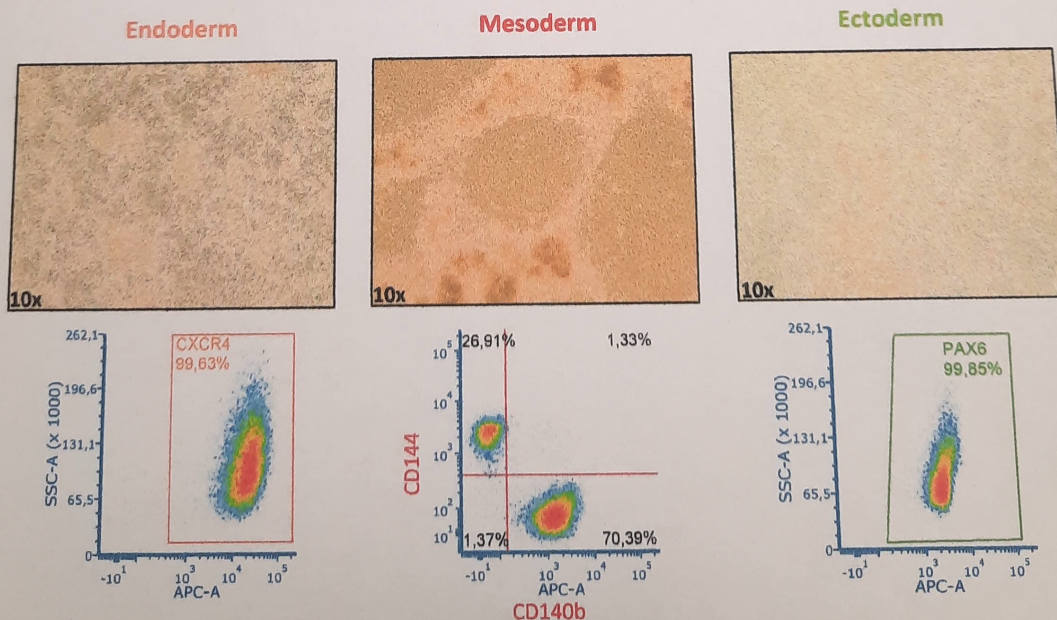


Figure 5: Trilineage differentiation. Top panel: Morphology of the iPSC OSR_003 #56 P14 on day 7 of culture in specific media for the differentiation in Endoderm (left), Mesoderm (middle) and Ectoderm (right). Magnification 10x.

Low panel: Dot plots of the percentage of cells positive for specific lineage markers (CXCR4 for Endoderm, CD140b and CD144 for Mesoderm and PAX6 for Ectoderm) 7 days after differentiation of iPSC OSR_003 #56 p14. Gate delimitates positive events.