Certificate of Analysis

CELL LINE NAME	**BIHi300-A**	hPSCreg Link: *https://hpscreg.eu/cell-line/BIHi300-A*
DONOR GENDER/AGE:	☐ Male ☒ Female ☐ unknown Age: 50-54	
TYPE OF DISEASE / GENETIC MODIFICATIONS	Breast cancer	
BANK	Master Bank, MB01, Passage 19, Freezing Date: 07.03.2024	
FREEZING METHOD	Bambanker	
CULTURE PLATFORM	Feeder Independent	
	Medium: E8	Coating: Geltrex
REPROGRAMMING	Sendai virus Vector details (e.g. Kit, Pub, AddgeneNr): CytoTune iPS 2.0	

TEST DESCRIPTION	Test Method	Test Specification	Result
STERILITY (viral pathogens)	☒ donor tested ☐ primary cells tested ☐ iPS clone tested	HBV, HCV, HIV negative	Pass
REPROGRAMMING VECTOR CLEARENCE	☐ parental cells tested ☐ antibody staining ☒ PCR	Vector not present	Pass
KARYOTYPE	CNV using SNP arrays	Result matches QC criteria	Pass
	G-Banding	Result matches expected karyotype	Pass
IDENTITY	STR Analysis	Identical to cells of origin	Pass
VIABILITY	Images of cells immediately post-thaw, at 48 hrs and at confluence	Growth to confluency typical of hPSCs	Pass
MORPHOLOGY	Light microscopy of cells	Typical morphology of undifferentiated hPSCs	Pass
STERILITY (mycoplasma)	Minerva Venor®GeM qOneStep	No contamination detected	Pass
STERILITY (bacteria/ yeast/ fungi)	Culture for 7 days in antibiotic free medium	No contamination detected	Pass
UNDIFFERENTIATED PHENOTYPE	Markers for undifferentiated hPSCs ☒ IF-Staining ☒FACS	Expression of at least three pluripotency markers detected	Pass
PLURIPOTENT DIFFERENTIATION POTENTIAL	directed differentiation	Successful differentiation to cells of all three germ layers	Pass
CONFIRMATION OF DISEASE GENOTYPE / EDITING	Sequencing of mutated site	Sequencing shows mutation	not aplicable

Date 20.08.2024

BIH Berlin Institute of Health @Charité	Stem Cell Core Unit **Report vector clearance of Sendai Virus (qPCR)**

Cell line name	BIHi300-A
Passage No.	13
Date of testing	16.02.2024
Protocol	8.4a Testing for remaining Sendai virus_CytoTune 2.0 via TaqMan (qPCR)
Samples	1. Negative control (H2O) 2. Positive control (C0474-1:10) 3. cDNA from sample RNA

Test

Sendi Virus vectors (SeV: all Sendi vectors, SeV Klf4, SeV cMyc, SeV KOS) presence was tested by qPCR according to Protocol 8.04a Testing for remaining Sendai virus CytoTune 2.0 via Taqman (qPCR). 45SRNA and GAPDH were used as positive amplification controls. The following CT value cut-offs were determined empirically.

Vector	CT value = positive sample	CT value = negative sample
SeV	≤30	>30
SeV-Klf4	≤35	>35
SeV-cMyc	≤33	>33
SeV-KOS	≤34	>34

Results

Sample	SeV CT	SeV-Klf4 CT	SeV-cMyc CT	SeV-KOS CT	Result
1.	UNDETERMINED	UNDETERMINED	UNDETERMINED	UNDETERMINED	Passed
2.	19,079	25,808	26,894	24,651	Passed
3.	UNDETERMINED	UNDETERMINED	UNDETERMINED	UNDETERMINED	SendiVirus Negative

Conclusion

The cell line is tested negative for Sendai virus.

Date 19.02.2024

Stem Cell Core Facility

Single Nucleotide Polymorphism (SNP)- Karyotype

	Reference			Cell line		
Sample (cell type, ID)	PBMC	44-19B		iPSC	BIHi300-A	
Passage No.				19		
Bank ID				MB01		
DNA sample ID	D0718			D0798		
Chip-ID and Position	208073030088, R06C01			208189810065, R02C02		
Date of testing	02.01.2024			15.04.2024		
Gender (provided/estimated from chip data)	Female	Female	√	Female	Female	√

Technology: Illumina BeadArray
Product: Illumina Infinium Global Screening Array-24 BeadChip
Manifest: GSAMD-24v3-0-EA_20034606_A1
Clusterfile: GSA-24v3-0_A1_ClusterFile

CNV Analysis
Algorithm: CNV-Partition
Version: 3.2.0

Parameters are set to detect copy number variations (CNVs) ≥ 45 kb and loss of heterozygosity (LOH) regions > 1 Mb with a confidence value > 35. Balanced translocations and inversions cannot be detected with this method. Aberrant copy number regions are identified by log R ratio and B allele frequency. Copy number changes (gains and losses) greater than **0.4 Mb** and regions of LOH above **5 Mb** are considered reportable and taken into account for interpretation. Genomic positions are based on genome build GRCh37/hg19.

If in the tested cell line (compared to the reference) new CNVs greater than **2 Mb** and/or LOH greater than **5 Mb** are detected the CNV QC test has "failed" regarding the internal QC criteria of CUSCO. We recommend not to use a "failed" cell line for further research or only after careful consideration.

Single Nucleotide Polymorphism (SNP)- Karyotype

Results

	BIHi300-A	Reference (44-19B)
call_rate	0.991	0.992
computed_gender	F	F
SNPs_post_filter	74.13 %	74.48 %
SNP.distance.to.ref	0	-
loss.gain_log2ratio	1.52	-1.81
total_calls_CNV	31	9
total_calls_LOH	15	8
reportable_new_calls_CNV	0	1
reportable_new_calls_LOH	1	0
critical_new_calls_CNV	0	0
critical_new_calls_LOH	0	0

Interpretation

- There was **1** new reportable LOH change identified in the iPSC line BIHi300-A.
  > A 2.759 MB LOH on chromosome 5 was observed.

The CNV analysis result suggests that the iPSC line contains neither CNVs > 2 Mb nor regions of LOH > 5 Mb. For more information, please find attached the HTML-Report.

Further information about genes in the detected regions and linked known diseases may be provided by the UCSC Genome Browser (https://genome.ucsc.edu) and Decipher (https://decipher.sanger.ac.uk/search).

References:

1. LaFramboise, T. (1 July 2009). "Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances". Nucleic Acids Research. 37 (13): 4181–4193.

2. Arsham, M. S., Barch, M. J., & Lawce, H. J. (Eds.) (2017). The AGT Cytogenetics Laboratory Manual (4th Ed.). Hoboken, NJ: John Wiley & Sons, Inc.

3. Haraksingh RR, Abyzov A, Urban AE. Comprehensive performance comparison of high-resolution array platforms for genome-wide Copy Number Variation (CNV) analysis in humans. BMC Genomics. 2017 Apr 24;18(1):321. doi: 10.1186/s12864-017-3658-x.

4. Wicell: https://www.wicell.org/home/characterization/cytogenetics/snp-microarray/single-nucleotide-polymorphism-snp-mircroarray-.cmsx

Stem Cell Core Facility

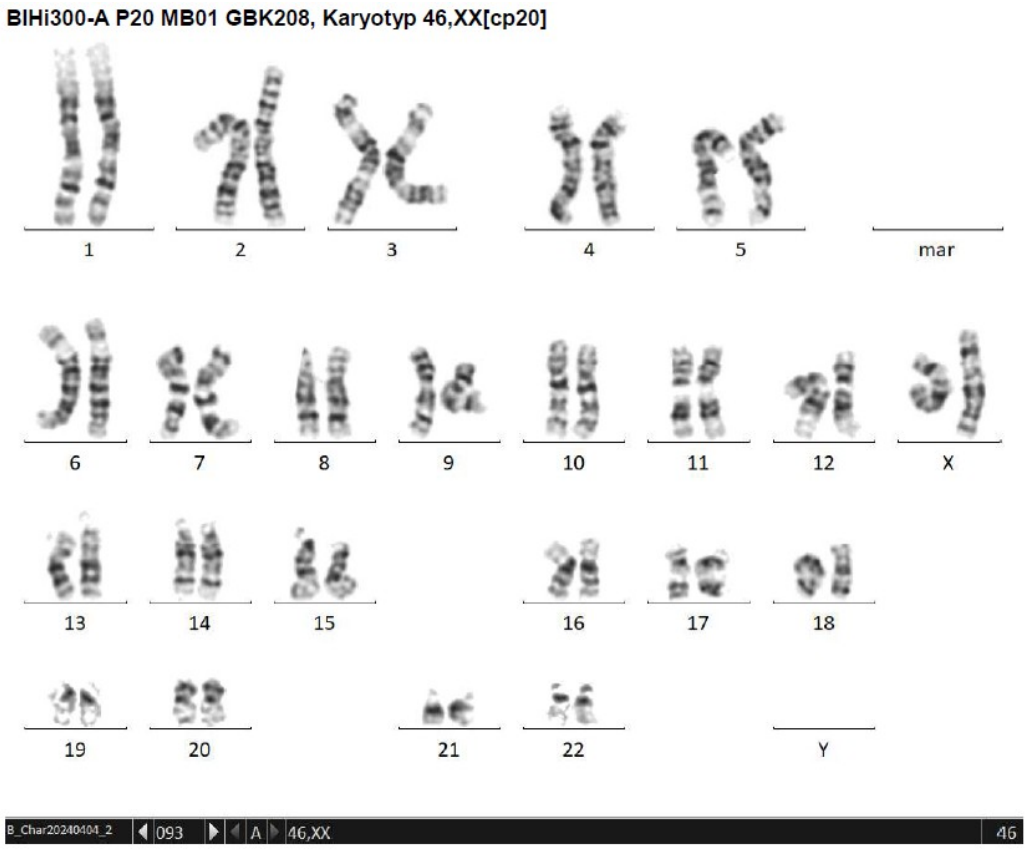
G-Banding - Karyotype

Cell line name	BIHi300-A
Bank ID	MB01
Passage No.	20
Date of testing	26.03.2024
Protocol	7.7 G-banded karyotyping

The sample preparation was carried out at BIH Stem Cell Core Facility and sent for G-banded-karyotyping to the “Institut für Humangenetik, Universitätsklinikum Jena”.

General comments: Karyotyping is performed using GTG stained metaphase chromosomes. With an average resolution of at least 200 bands per haploid chromosome set. Sub-microscopic changes (microdeletions/duplications) and changes <10Mb cannot be excluded by this method. Mosaics in the form of clonal changes are reported when the same change or chromosome gain occurs more than twice, and chromosome losses occur more than 3 times. A composite karyotype (cp) from 20 metaphase plates in the currently valid ISCN nomenclature is reported and a representative karyogram is provided

Results



Conclusion:

A normal female karyotype 46XX was detected for the examined sample.

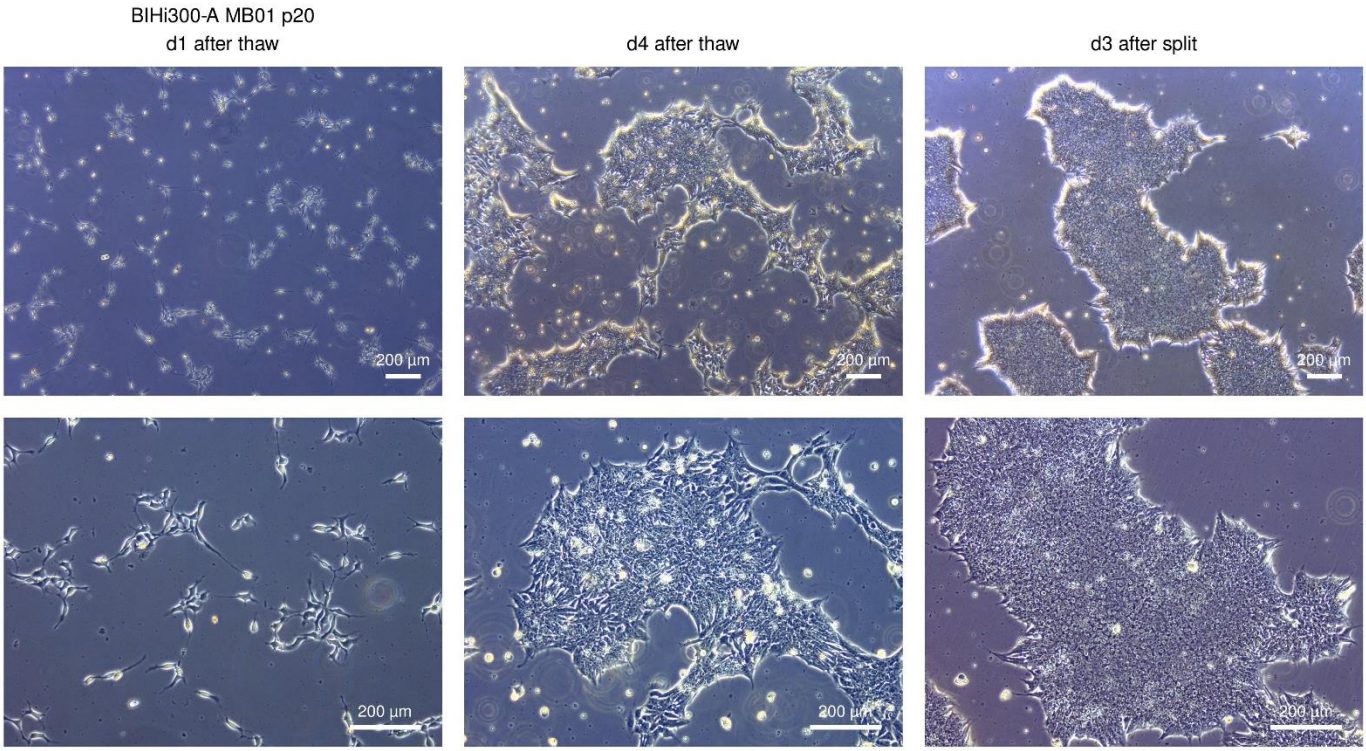
Date: 11.04.2024

BIH Berlin Institute of Health @Charité	Core Unit Pluripotent Stem Cell and Organoids (CUSCO) **Morphology and Viability**

Cell line name	BIHi300-A
Bank ID	MB01
Passage No.	20
Date of testing	23.03.2024
Coating / Medium	Geltrex / mTeSR

One vial of the cell bank was thawed and monitored during antibiotics-free cultivation. ROCK Inhibitor was used during the first 24 hours only. Cultures were evaluated regarding their morphology and viability.

Images:



Conclusion:

Cells show a good post-bank recovery after thawing and form colonies exhibiting typical morphology of undifferentiated hPSCs.

Date: 11.04.2024

BIH Berlin Institute of Health @Charité	Stem Cell Core Facility **Sterility (Mycoplasma, Bacteria/Yeast/Fungi)**

Cell line name	BIHi300-A
Bank ID	MB01
Passage No.	20
Test date	09.04.2024
Protocol	8.1.3 Mycoplasma testing_qPCR Minerva
Samples	1: Negative Control (culture medium of Cell Line tested) 2: Positive Control (Mycoplasma DNA from *Venor® GeM qOneStep Kit*) 3: Cell culture supernatant from cell line

Bacteria/Yeast/Fungi

Test

Cells were cultured without the addition of antibiotics over a period of 7 days. Cultures were checked daily for growth of bacteria, yeast and fungi by microscopy.

Results

No turbidity of the cell culture medium or microbial colonies were detected.

Mycoplasma

Test

Cells were cultured without the addition of antibiotics to a confluency of 80-90%. Mycoplasma contamination was tested by the qPCR-based *Venor®GeM qOneStep Kit*. Mycoplasma are detected at 520 nm by amplifying the 16S rRNA coding region in the mycoplasma genome. False-negative results caused by PCR inhibition are identified by the internal amplification control, detected at 560 nm.

Mycoplasma 520 nm	Internal amplification control 560 nm	Interpretation
Ct<40	Irrelevant	Sample is Mycoplasma contaminated
Ct≥40	Ct≥40	qPCR inhibition
Ct≥40	Ct<40	Sample is Mycoplasma free

Results

Sample	Ct of Mycoplasma DNA	Ct of Internal amplification DNA	Result
1 (neg. control)	>45	22,384	*Passed*
2 (pos. control)	22,082	24,624	*Passed*
3	>45	9,773	***Negative***

Conclusion

The cell line was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Date: 15.04.2024

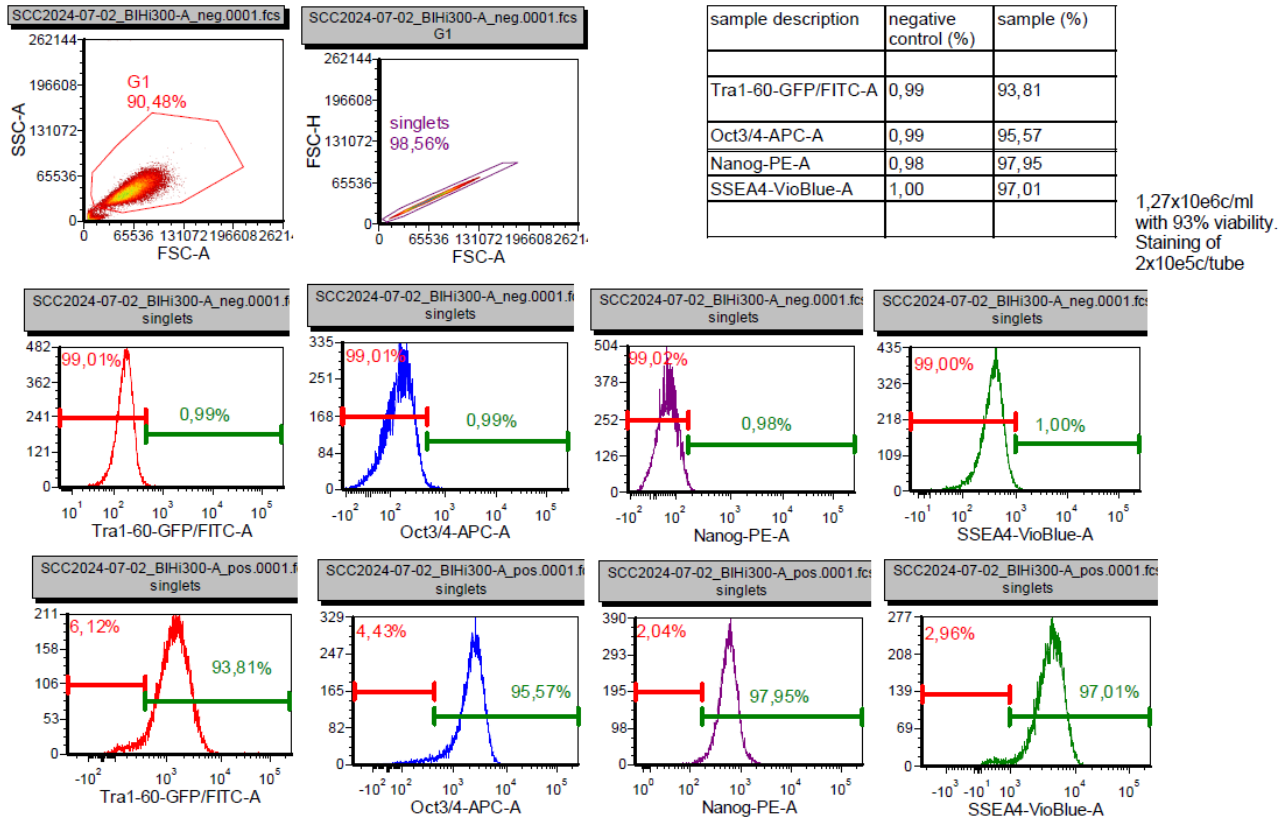
Stem Cell Core Unit

FACS analysis of markers in undifferentiated hPSCs

Cell line name	BIHi300-A
Bank ID	MB01
Passage No.	20
Date of testing	02.07.2024
Protocol	7.14 FACS analysis of pluripotency markers

Results

20240702_FACS analysis of markers of undifferentiated BIHi300-A MB01 p20_one sample stained with all antibodies



sample description	negative control (%)	sample (%)
Tra1-60-GFP/FITC-A	0,99	93,81
Oct3/4-APC-A	0,99	95,57
Nanog-PE-A	0,98	97,95
SSEA4-VioBlue-A	1,00	97,01

1,27x10e6c/ml with 93% viability. Staining of 2x10e5c/tube

Conclusion

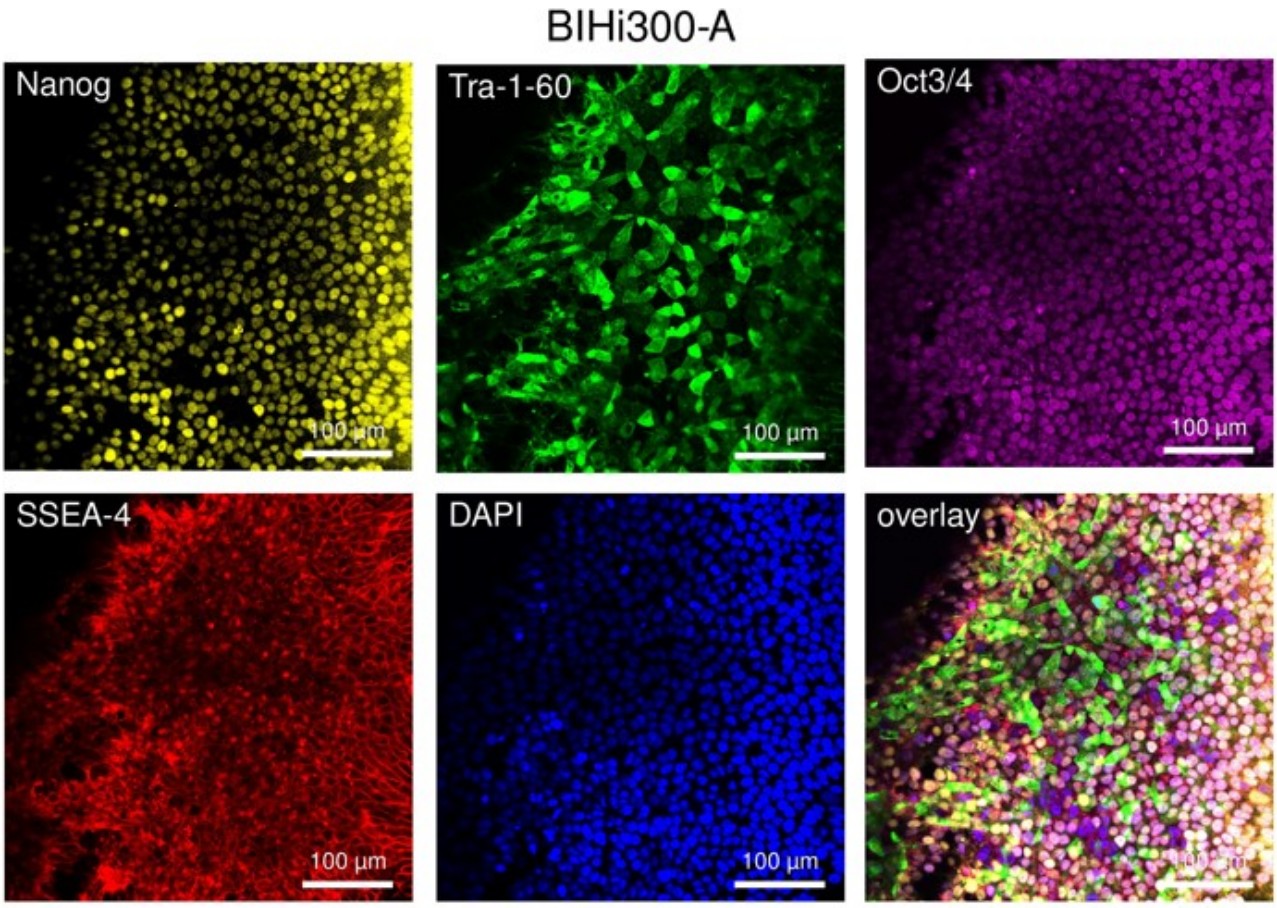
The cell line shows positive FACS results (over 80% positive) for the tested undifferentiated stem cell markers Tra1-60, OCT3/4, NANOG and SSEA-4.

Date: 02.07.2024

Immunofluorescence staining of markers for undifferentiated hPSCs

Cell line name	BIHi300-A
Bank ID	MB01
Passage No.	22
Date of testing	04.04.2024
Protocol	7.1 Immunofluorescence staining of markers for undifferentiated cells

Results:



Conclusion:

The cell line shows positive staining results for the tested undifferentiated stem cell markers Nanog, OCT3/4, Tra-1-60 and SSEA4.

Stem Cell Core Facility

Validation of pluripotent differentiation potential

Cell line name	BIHi300-A
Bank ID	MB01
Passage No.	24
Date of testing	13.08.2024
Protocol	7.03.0 Validation of pluripotency capacity by Trilineage differentiation with Miltenyi kit

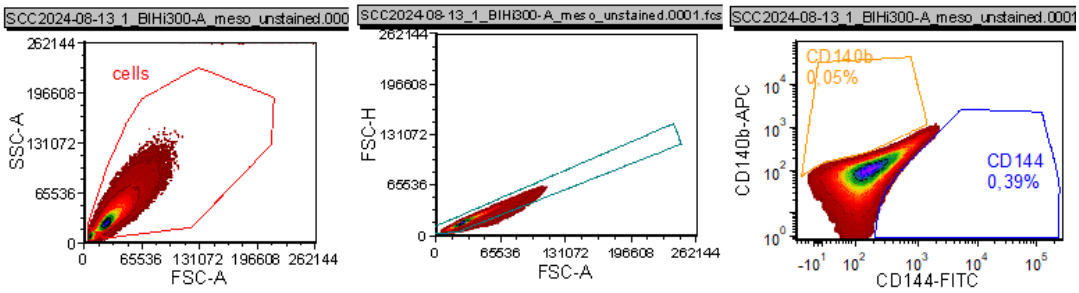
Method

Test was performed regarding the StemMACS Trilineage Differentiation Kit, human (MACS Miltenyi Biotec, Cat-No. 130-115-660). The 7-day assay enables direct differentiation of pluripotent stem cells into ecto-, meso and endoderm. The resulting cell population was measured by FACS analysis.

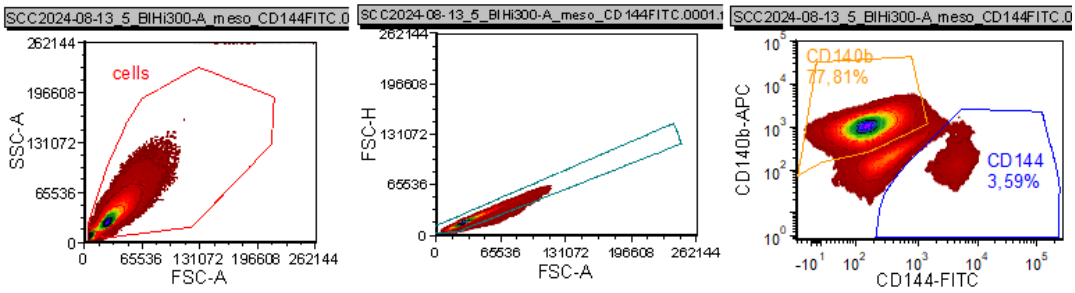
Result

20240813 Trilineage with BIHi300 MB01 p24 mesoderm differentiation with StemMACS Tril. Diff. Kit

negative control

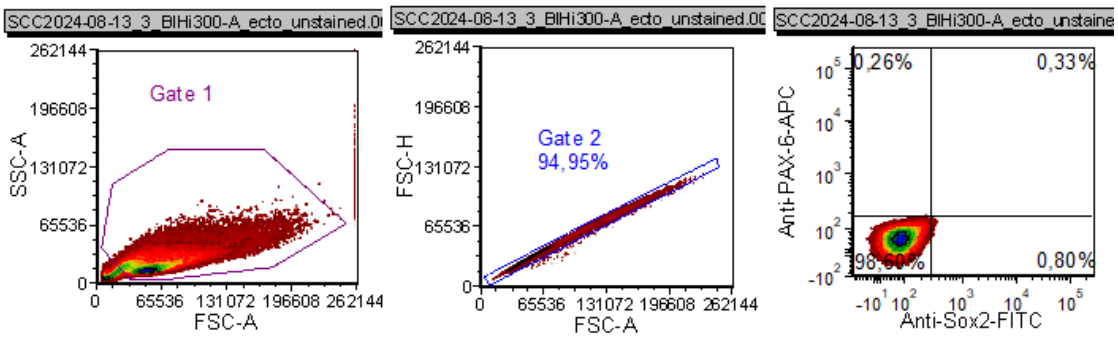


CD140b-APC, human CD144(VE-Cadherin)-FITC, human

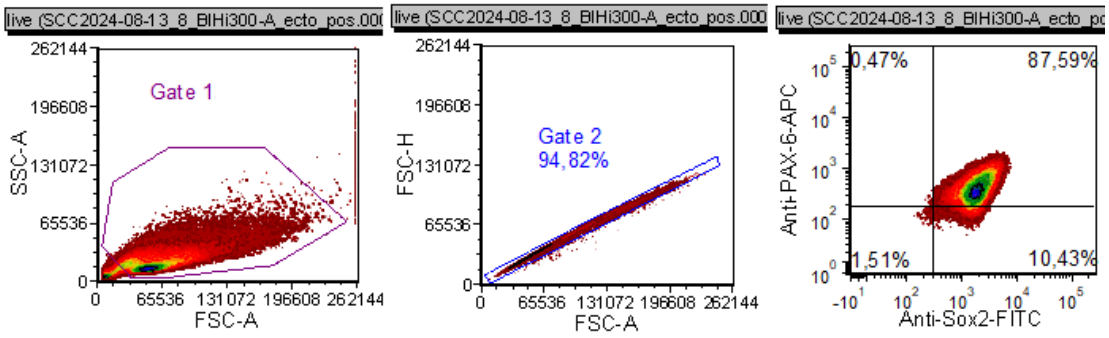


20240819 Trilineage with BIHi300-A MB01 p24 ectoderm differentiation with StemMACS Tril. Diff. Kit

negative control

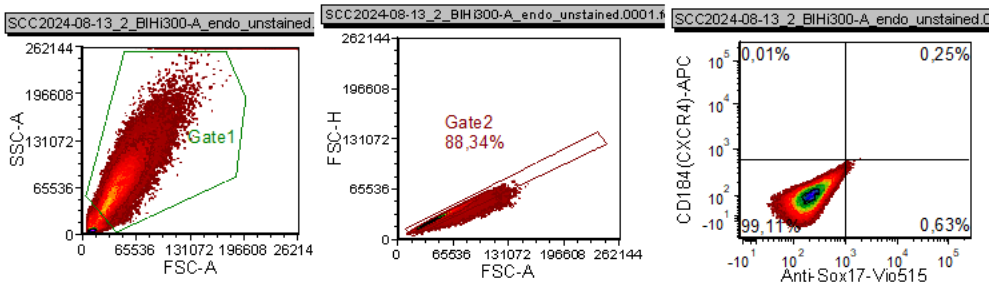


Anti-PAX-6-APC, human
Anti-Sox2-FITC, human

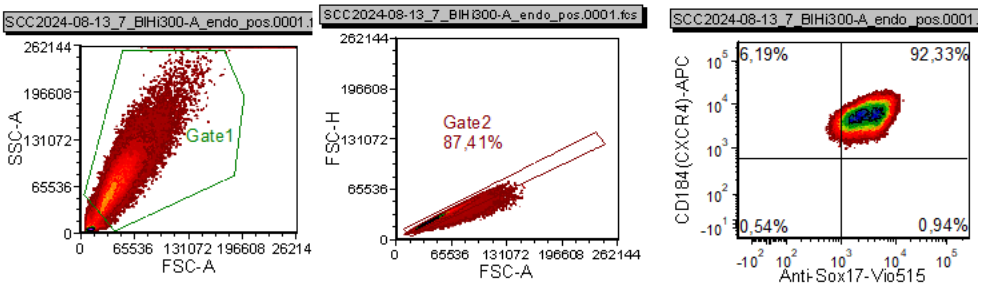


20240819 Trilineage with BIHi300-A MB01 p24 endoderm differentiation withStemMACS Tril. DIff. Kit

negative control



CD184 (CXCR4)-APC Anti-Sox17-Vio515, human



Conclusion

The cell line shows potency to differentiate into mesoderm, ectoderm and endoderm lineages. The lineage markers CD140b, CD144 (Mesoderm), Sox2, Pax6 (Ectoderm) and Sox17, CD184 (Endoderm) showed positive FACS results.

Date: 19.08.2024