Certificate of Analysis

BIH STEM CELL CORE

CELL LINE NAME	**BIHi264-A**	hPSCreg Link: *https://hpscreg.eu/user/cellline/BIHi264-A*
DONOR GENDER/AGE:	☐ Male ☒ Female ☐ unknown Age: 51	
TYPE OF DISEASE / GENETIC MODIFICATIONS	**Patient who received chemotherapy - severe neuropathy**	
BANK	Master Bank, ID MB01. , Passage 13, Freezing Date: 13.12.2019	
FREEZING METHOD	Bambanker	
CULTURE PLATFORM	Feeder Independent	
	Medium: Homemade 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)	☒ Blood screening Donor ☐ PCR (primary cells) ☐ PCR (iPS clone)	HBV, HCV, HIV negative	Pass
REPROGRAMMING VECTOR CLEARENCE	☒ PCR ☐ AB staining	Vector not present	Pass
KARYOTYPE	CNV using SNP arrays	No significant changes compared to cells of origin	Pass
	G-Banding	No significant changes compared to cells of origin	Pass
IDENTITY	STR Analysis	Identical to profile of primary cells	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
	Pluritest	Pluripotency and Novelty Scores above threshold	Pass
PLURIPOTENT DIFFERENTIATION POTENTIAL	directed differentiation	Successful differentiation to cells of all three germ layers	Pass

date / initials: 21.12.21 / [redacted]

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Kompetenz von Charité und Vivantes

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10117 Berlin

Endbefund

14-18B-V3 (w) BIHi264
Geb. 00.00. 0
Fall: 11460753

Auftrag: 2725120
Abnahme: 27.05.2019 14 00
Eingang: 08.07.2019 17:29

Untersuchung	Ergebnis	Einheit	Referenz (w)	Grafik
Infektionsdiagnostik				
Material: Serum				
Hepatitis & HIV				
HBs-Antigen im Serum	negativ			
HBc-Antikörper im Serum	negativ			
HBs-Antikörper im Serum	negativ Anti-HBs-Bewertung (Fa.Roche cobas): <10 IE/l negativ, Immunität nicht protektiv	IU/l		
HBV-Serologiebefund	z.Zt. kein serologischer Hinweis auf akute, chronische oder zurückliegende HBV-Infektion.			
Hep.C-Virus AK im Serum	negativ Kein serologischer Hinweis auf eine Hepatitis C. Zur Erfassung der frühen Phase der Hepatitis C ist eine HCV-RNA Bestimmung erforderlich.			
HIV1/2-AK ,P24-AG	negativ Im Screeningtest (Fa. Roche) wurden weder HIV-1 oder HIV-2 spezifische Antikörper noch HIV1/2 p24-Antigen nachgewiesen.			

° Die Untersuchung wurde in einem Auftragslabor durchgeführt und durch den dor igen Laborarzt medizinisch validiert. °° Methode nicht akkreditiert

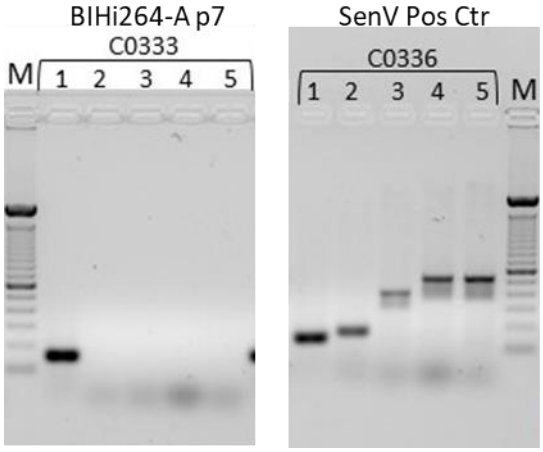
BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Report Sendai Virus Testing**

Cell line name	BIHi264-A
Passage No.	7
Bank	Seed
Name operator	[redacted]
Date of testing	12.11.2019
Protocol	8.4. Testing for remaining Sendai virus_CytoTune 2.0
Sample	A: BIHi264-A B: C0336 SenV positive control

Results

1,5 % standard agarose gel with DNA stain Ethidiumbromid 7µL/400 mL

PCR picture:



Primer

1	Hu18sRNA OL0107/8	152 bp
2	SeV OL0109/10	181 bp
3	SeV_Klf 4 OL0111/2	410 bp
4	SeV_cMyc OL0113/4	532 bp
5	SeV_KOS OL0115/6	528 bp

PCR Results - Conclusion

The cell line BIHi264-A at passage 7 was tested negative for Sendai virus.

Responsible person / date: [redacted] / 12.11.2019

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

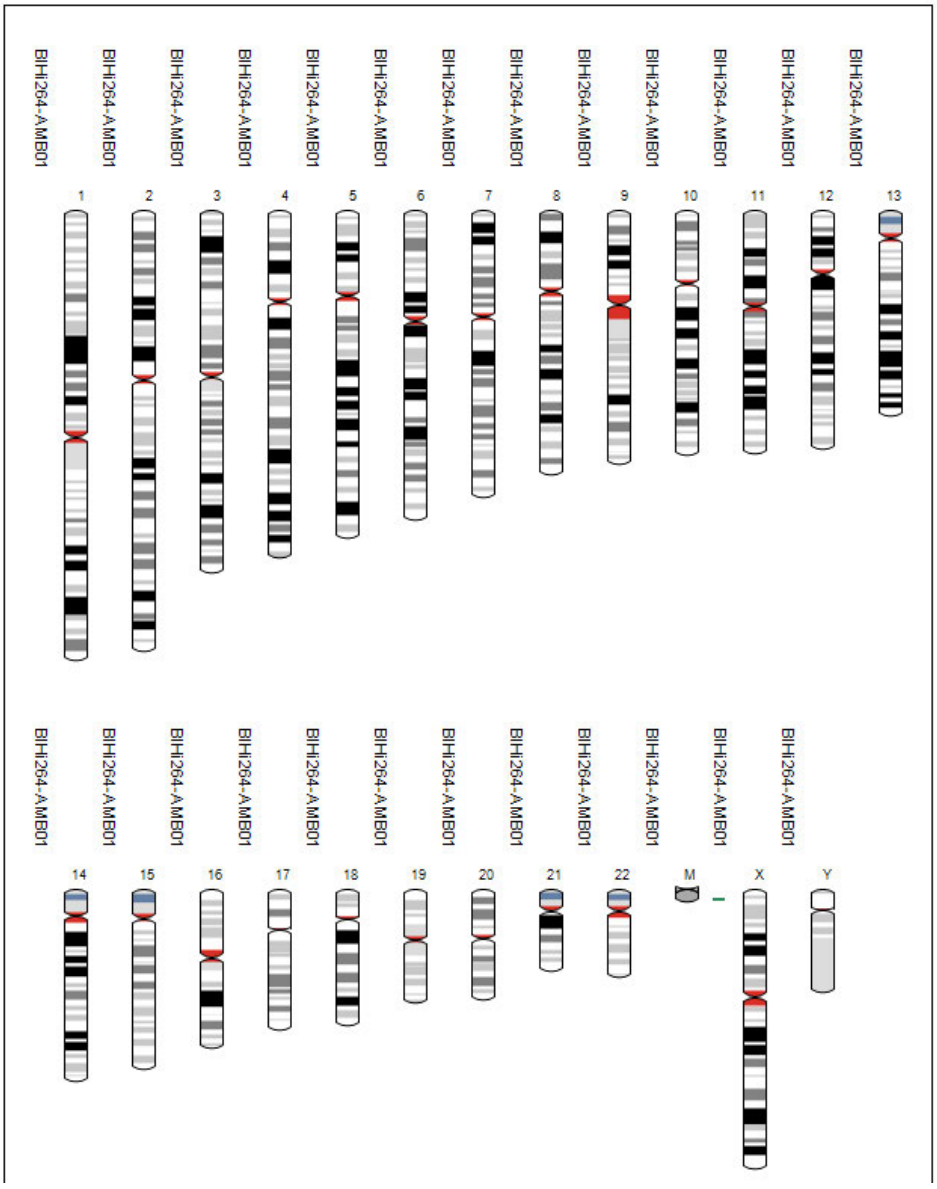
Single Nucleotide Polymorphism (SNP)- Karyotype

Cell line name	BIHi264-A MB01
Passage No.	13
Date of testing	25.02.2020

IPSC´s where karyotyped using the Illumina platform and the OMNI-EXPRESS-8v1.6 Chip. The Analysis was done using Karyostudio 1.3.

Virtual Karyotype:

Gain (Area marked in green), Loss (Area marked in red), Loss of heterozygosity (Area marked in gray), MB (mega bases)



Single Nucleotide Polymorphism (SNP)- Karyotype

Conclusion:

Based on the gender prediction of genome studio the BIHi264-A cell line is a female iPSC line. The cell line BIHi264-A has a normal karyotype showing no larger areas of insertions or deletions.

Some of the changes can´t be detect using conventical techniques to analyze the karyotype stability like G-Banding (LOH).

Responsible person / date: [redacted] / 09/03/2020

Attachments:

Cytogentics Report
Table of affected genes

Praxis für Humangenetik
Beratung & Diagnostik

Zertifiziert bis 12/2017

Fachärzte/Innen für Humangenetik
Prof. Dr. med. Gundula Kadgien*
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[redacted]
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13353 Berlin

Zytogenetische Untersuchung von Zelllinien

Sehr geehrter Herr Kollege,

wir berichten über die Passage 16 der Zelllinie SZ_BIHi264-A MB01.

Analytik: Chromosomenanalyse nach GTG-Bänderung

Anzahl der ausgewerteten Metaphasen pro Passage:	20
Anzahl der Karyogramme pro Passage:	5
Banden nach GTG	400-450

Ergebnis: **46,XX**

Interpretation:
In den untersuchten Mitosen wurde ein diploider weiblicher Chromosomensatz mit 46 Chromosomen ermittelt.
Bei der erreichten Bandenauflösung ergab sich kein Hinweis auf klonale strukturelle bzw. numerische Chromosomenaberrationen.
Mit dieser Untersuchung sind nur lichtmikroskopisch sichtbare Veränderungen an den Chromosomen erfasst. Der Ausschluss schwacher Mosaike ist aus methodischen Gründen prinzipiell nicht möglich. Veränderungen an einzelnen Genen (Genmutationen) oder andere Störungen sind mit dieser Methode nicht nachweisbar.

Weiterführende Untersuchungen sind nach Absprache möglich.

Befund vom 19.03.2020

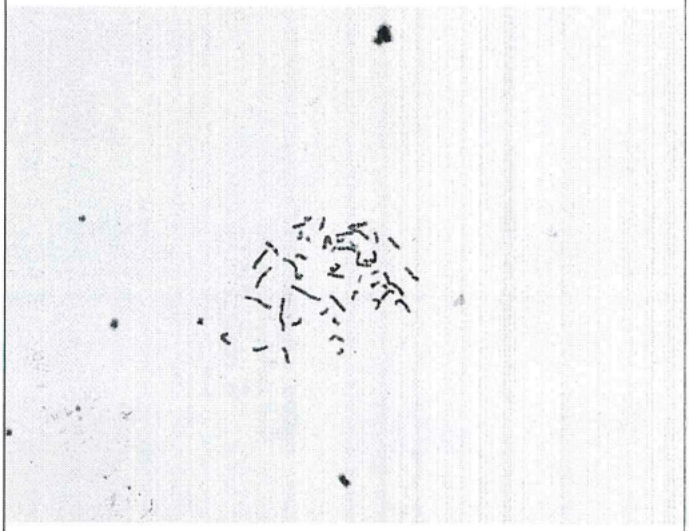
Mit kollegialen Grüßen

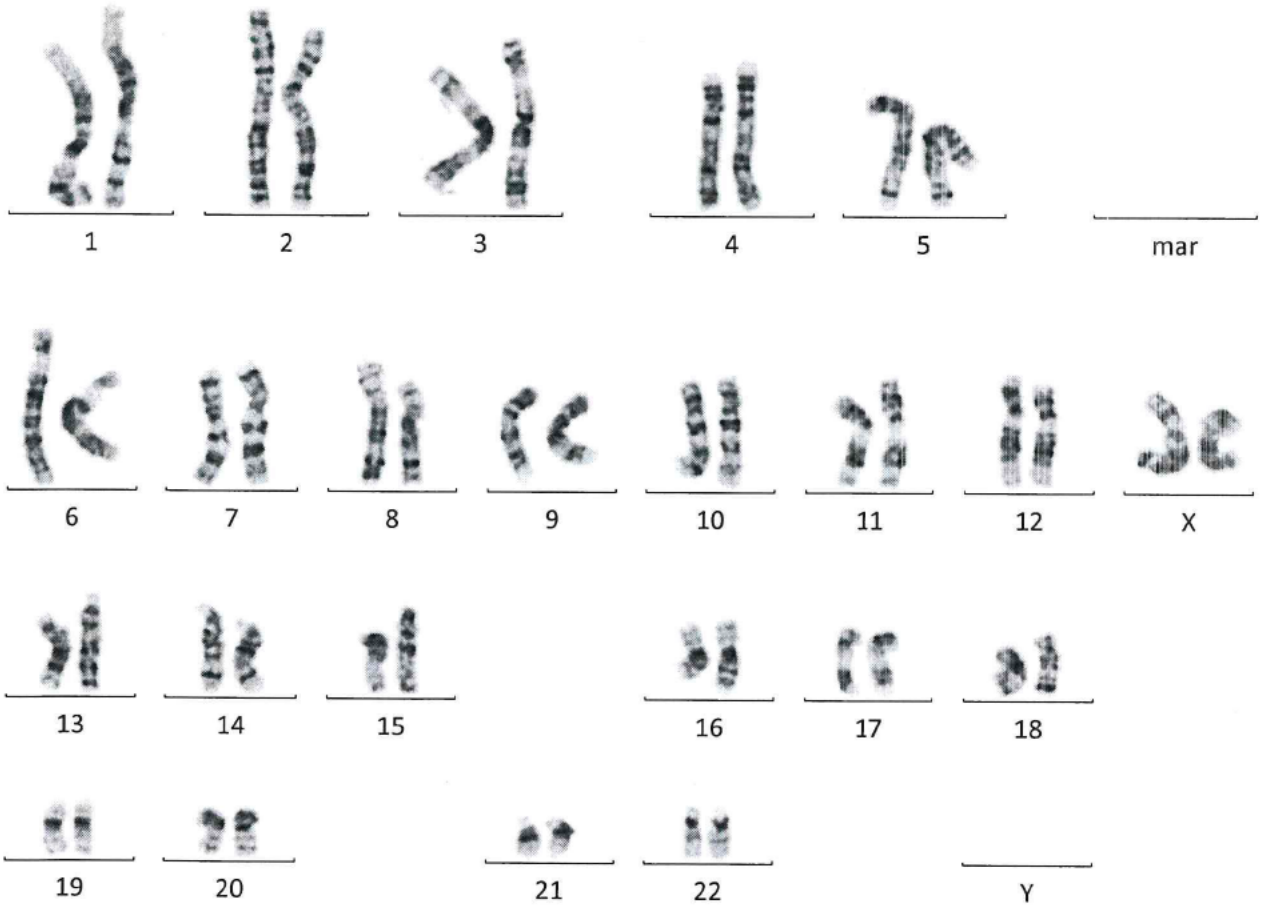
[redacted]

Praxis für Humangenetik

Dr. med. [redacted] - Fachärztin für Humangenetik

Friedrichstraße 147 - 10117 Berlin - Tel.: [redacted]



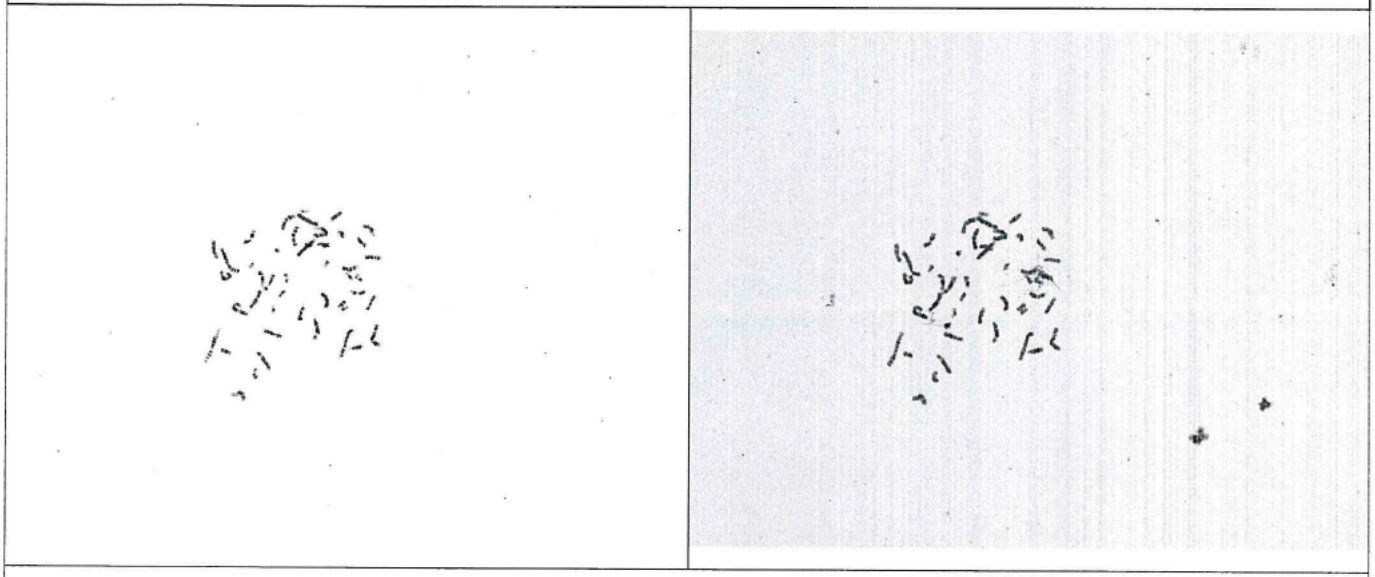


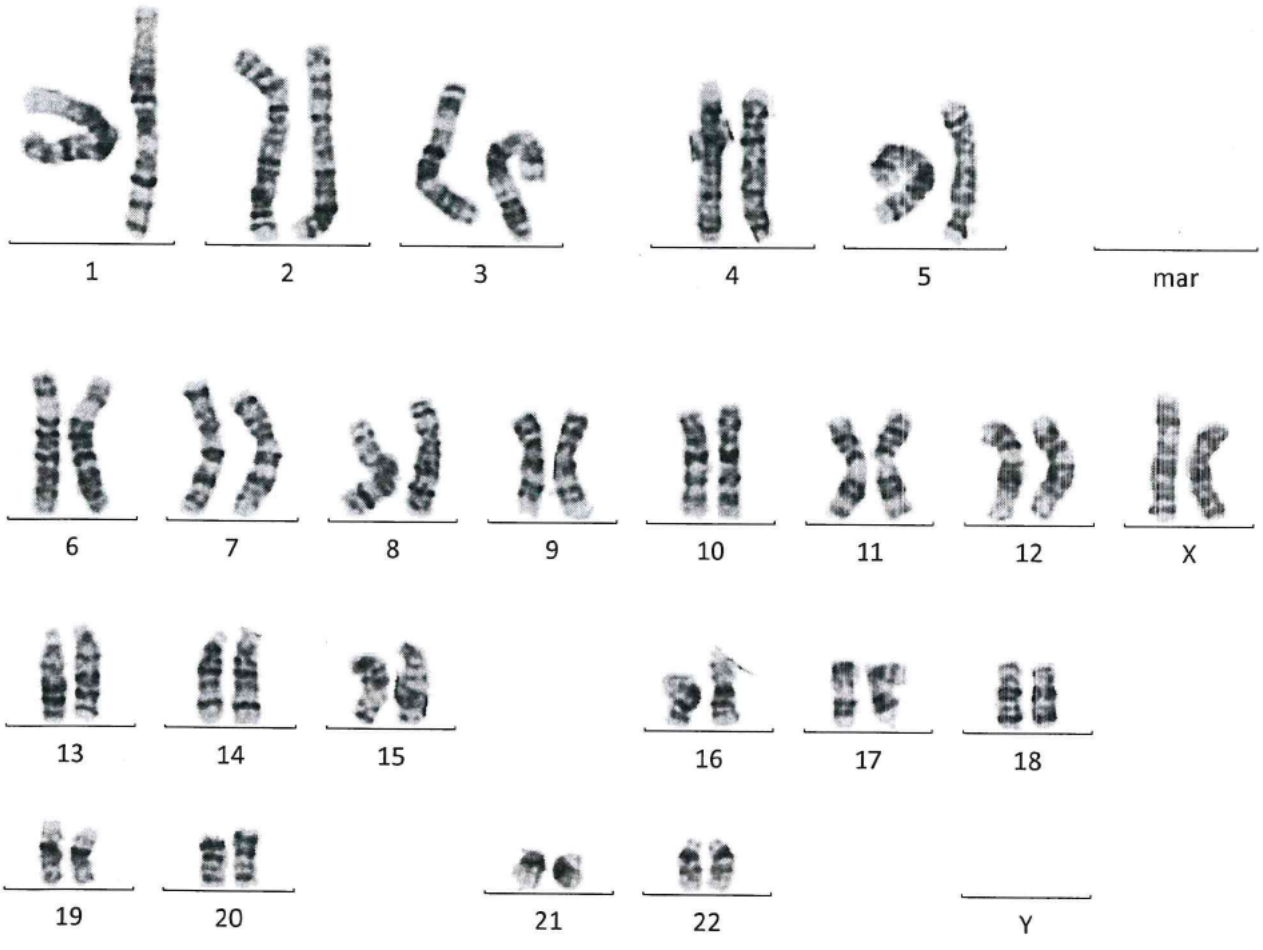
SZ BIHi264-A MB01 • Cu p16 • Sl II • 026 46,XX

Praxis für Humangenetik

Dr. med. [redacted] Fachärztin für Humangenetik

Friedrichstraße 147 - 10117 Berlin - Tel.: [redacted]





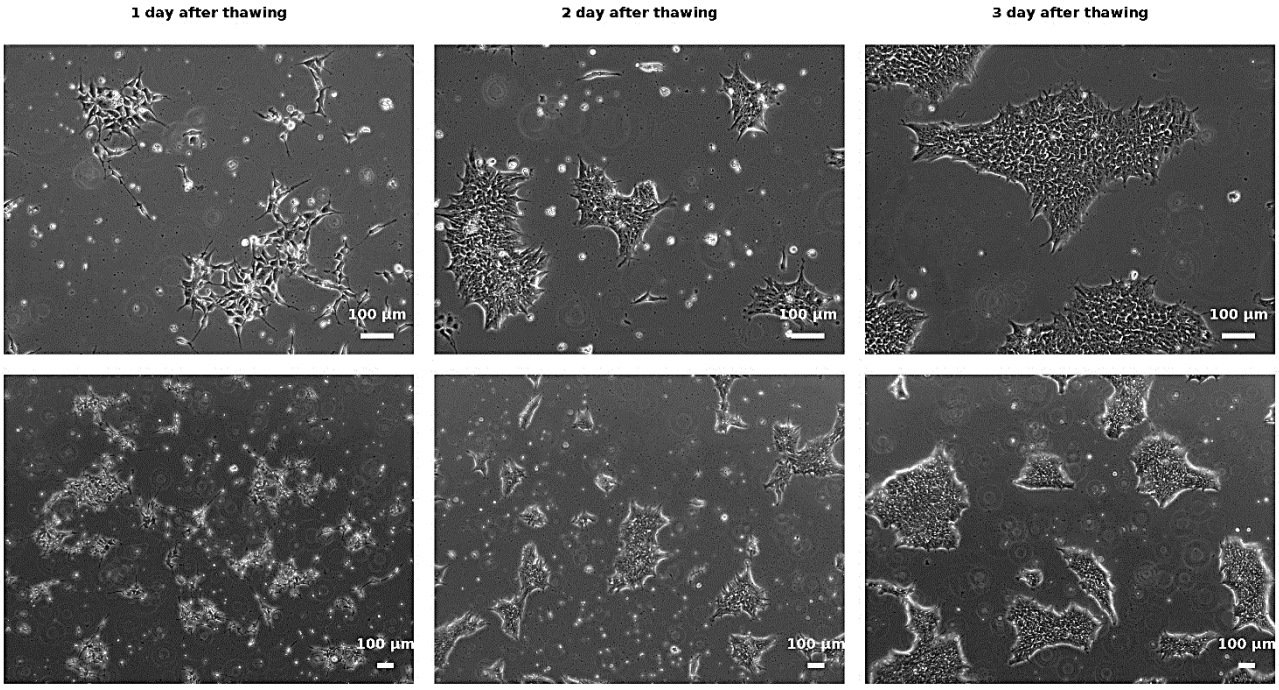
SZ BIHi264-A MB01 • Cu p16 • Sl II • 038 46,XX

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Morphology and Viability**

Cell line name	BIHi264-A
Passage No.	14
Bank	Master bank
Name operator	[redacted]
Date of testing	25.02.2020

An aliquot of the master cell bank was thawed up and monitored during antibiotics-free cultivation with E8 Medium. ROCK inhibitor was used only during the first 24 hours.

Images:



Conclusion:

Cells from the cell bank show a good post-bank recovery after thawing and getting confluent within one week.

The cell line BIHi264-A shows typically morphology of undifferentiated hPSC.

Responsible person / date: 25.02.2020 / [redacted]

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Sterility (Mycoplasma, Bacteria/Yeast/Fungi)**

Cell line / Passage No.	BIHi264-A / p24
Cell bank	MB01
Operator name	[redacted]
Test date	18.02.2020
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	29,1	Passed
2 (pos. control)	27,7	29,3	Passed
3	>45	28,9	**Negative**

Conclusion

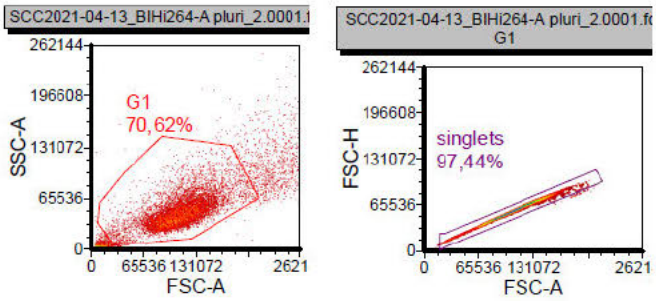
The cell line was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Responsible person / date: [redacted] / 24.02.2020

BIH Berlin Institute of Health @Charité	Stem Cell Core Unit **FACS analysis of markers in undifferentiated hPSCs**

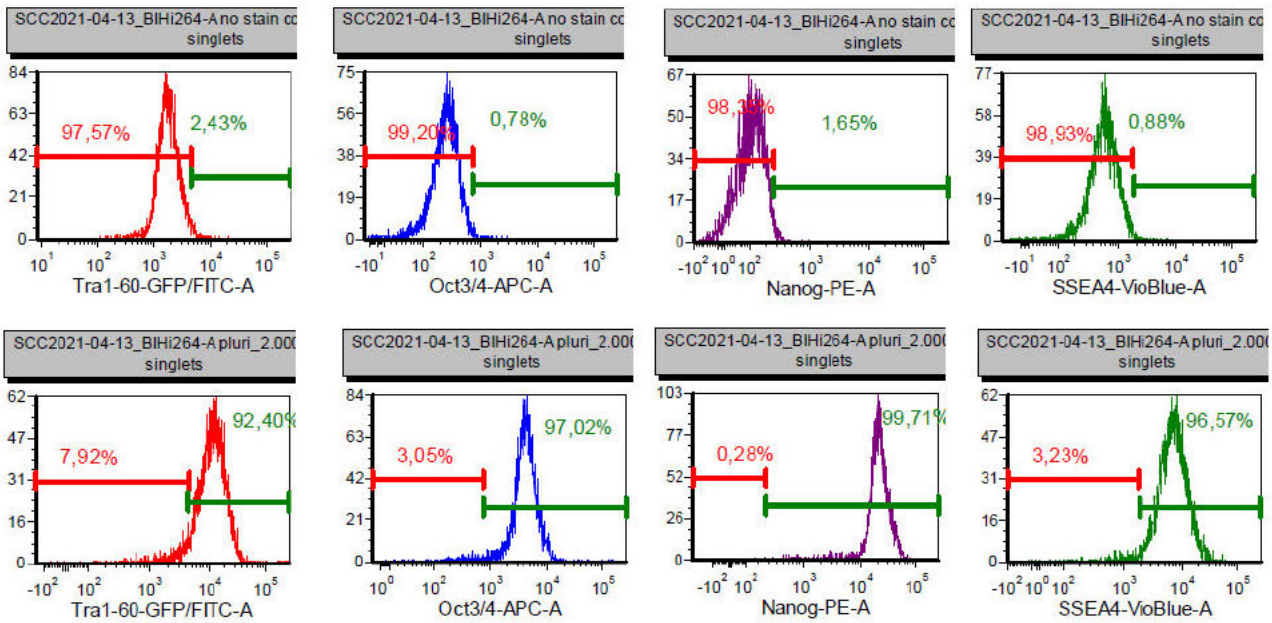
Cell line name	BIHi264-A
Bank ID	MB01
Passage No.	P15
Date of testing	14.04.2021
Protocol	7.14 FACS analysis of pluripotency markers

20210414_[redacted]FACS analysis of markers of undifferentiated BIHi264-A MB01 p15



sample description	negative control (%)	sample (%)
Tra1-60-GFP/FITC-A	2,43	92,40
Oct3/4-APC-A	0,78	97,02
Nanog-PE-A	1,65	99,71
SSEA4-VioBlue-A	0,88	96,57

2 well of 6 WP: 4,22x10e5c, 79% viability,
Staining of 3x10e5c/tube



The cell line BIHi264-A at passage 15 shows positive FACS results (over 80% positive) for the tested undifferentiated stem cell markers Tra1-60, OCT3/4, NANOG and SSEA-4.

Initials / date [redacted] / 04.05.2021

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

Validation of pluripotent phenotype (PluriTest)

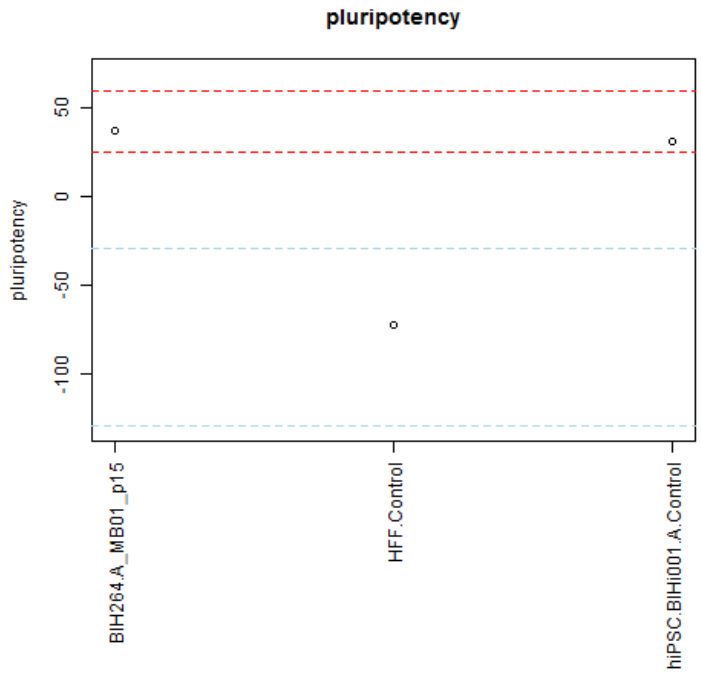
cell line name	BIHi264-A p15
Bank	Masterbank (MB01)
Date of testing	15.07.2020
Sample	A: BIHi264-A B: HFF (human foreskin fibroblasts, negative control) C: BIHi001-A (human hiPSC line, positive control)

The gene expression profiles of the samples were measured using Illumina HT12v4 gene expression arrays. The generated data was analyzed using the PluriTest bioinformatic assay for pluripotency (https://www.pluritest.org/)

Results

	pluri-raw	pluri logit-p	novelty	novelty logit-p	RMSD
BIH264.A_MB01_p15	37.84	1.00	1.53	0.03	0.44
HFF.Control	-72.18	0.00	3.44	1.00	0.86
hiPSC.BIHi001.A.Control	31.32	1.00	1.50	0.02	0.44

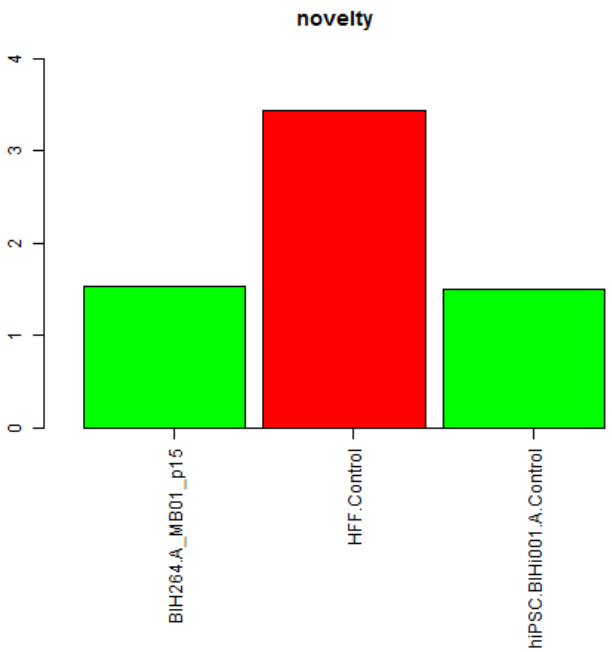
Model-Based Multi-Class Pluripotency Score:



A score that is based on all samples (pluripotent cells, somatic cells and tissues) in the stem cell model matrix. Samples with positive values are more similar to the pluripotent samples in the model matrix than to all other classes of samples in the matrix. The area between the red lines indicates the range that contains approximately 95 percent of the pluripotent samples tested. The *Pluripotency Score* gives an indication if a sample contains a pluripotent signature, but not necessarily if the cell preparation is a normal, bona-fide hESC or iPSC.

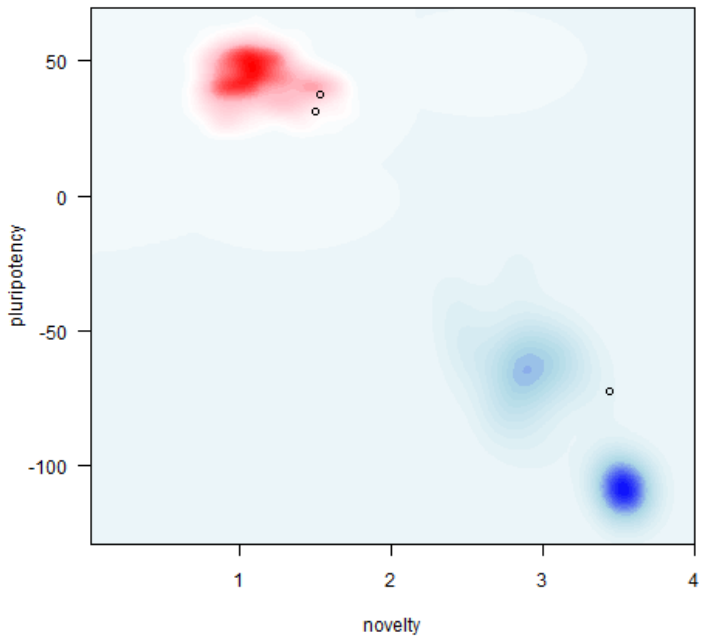
Validation of pluripotent phenotype (PluriTest)

Novelty score:



A score that is based on well-characterized pluripotent samples in the stem cell model matrix. Samples are color-coded green (pluripotent), orange, red (not-pluripotent) based on the probabilities given from the logistic regression model. Orange and red samples are more dissimilar to the pluripotent samples in the model matrix than the other pluripotent samples in the matrix. A low Novelty Score indicates that the test sample can be well reconstructed based on existing data from other well-characterized iPSC and ESC lines.

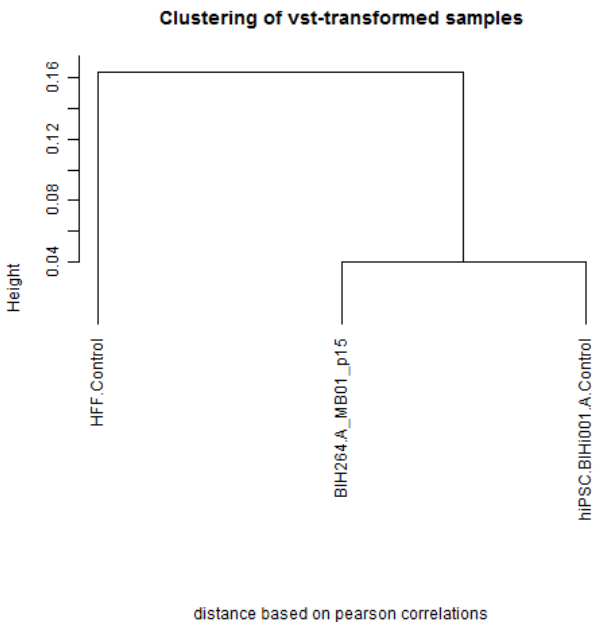
Overview:



Combines the Pluripotency Score on the y-axis with the Novelty Score on the x-axis. The red and blue background hint to the empirical distribution of the pluripotent (red) and non-pluripotent samples (blue) in the test data set.

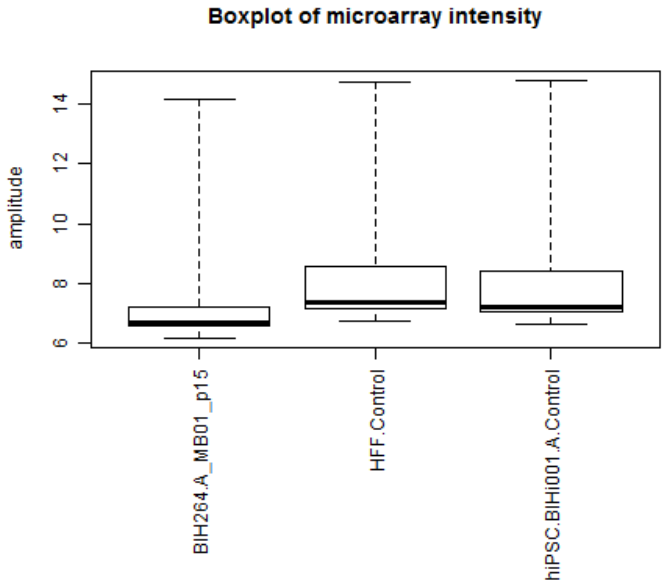
Validation of pluripotent phenotype (PluriTest)

Quality Control: Hierarchical Clustering:



A plot generated by the Lumi package after the samples were transformed with a variance stabilizing transformation (VST) and before robust spline normalization (RSN). Outlier arrays with too much technical variation might be spotted if they do not cluster with their respective technical or biological replicates from the same sample or sample type.

Quality Control: Boxplots



A plot generated by the Lumi package after the samples were transformed with a variance stabilizing transformation (VST) and before robust spline normalization (RSN). Outlier arrays with too much technical variation might be spotted if they show a different probe intensity distribution pattern in the box-plots when compared to the other arrays on the same chip or when compared to arrays on other chips.

Conclusion

The cell line BIHi264-A at passage 15 passed the PluriTest assay for pluripotency.

Responsible person / date: [redacted] / 03.09.2020

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

Validation of pluripotent differentiation potential

Cell line / Passage No.	BIHi264-A P18
Cell bank	MB01
Name operator	[redacted]
Date of testing	02.07.2020

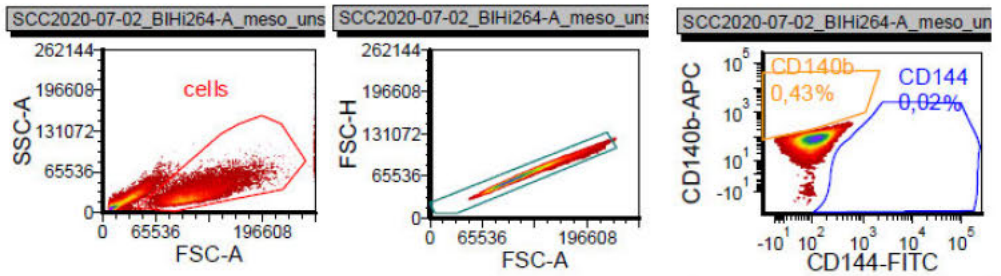
Method

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 has been measured by FACS analysis.

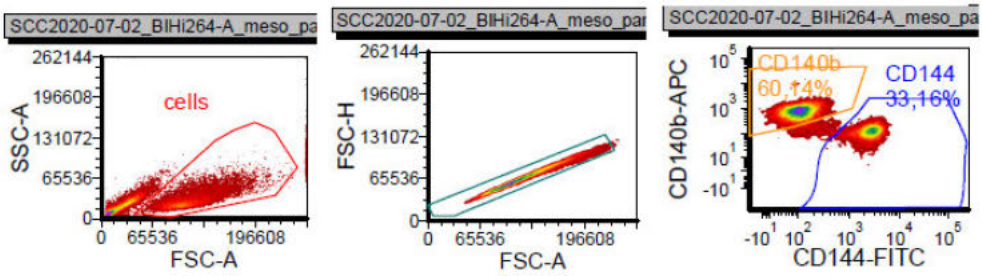
Result

20200702 [redacted]rilineage with BIHi264-A mesoderm differentiation

negative control



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



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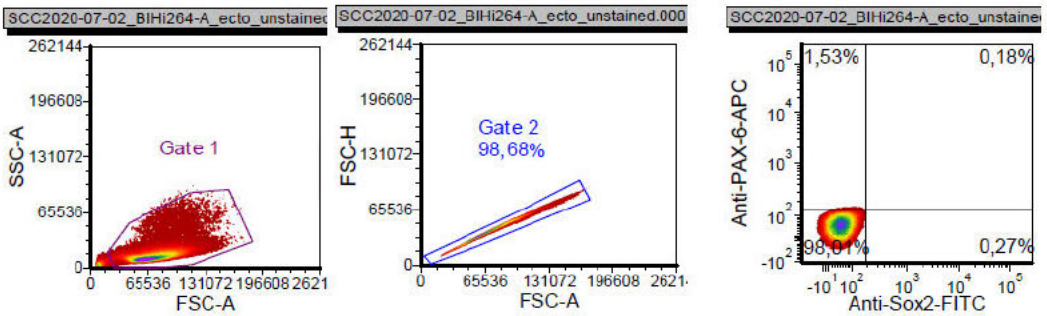
Stem Cell Core Facility

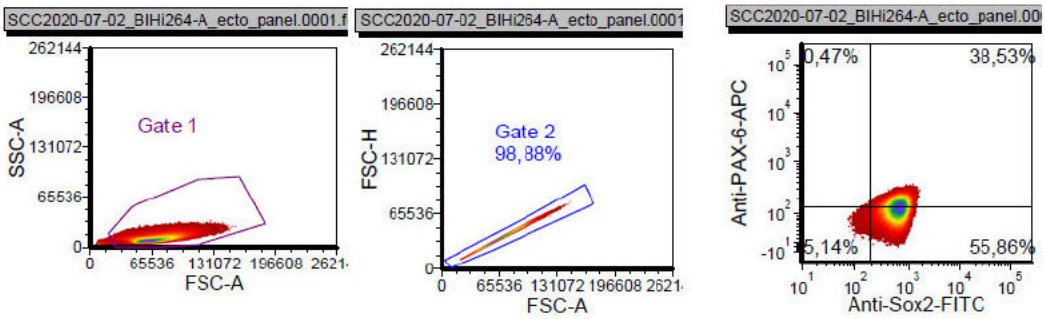
Validation of pluripotent differentiation potential

20200702 [redacted] Trilineage with BIHi264-A ectoderm differentiation

negative control

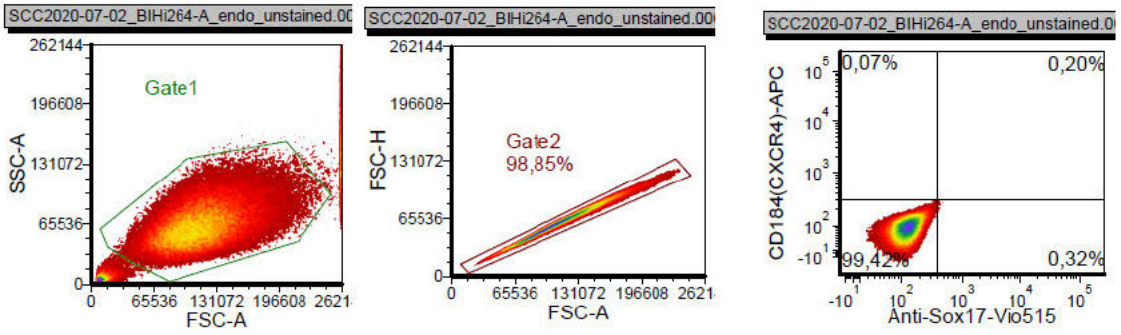


Anti-PAX-6-APC, human
Anti-Sox2-FITC, human

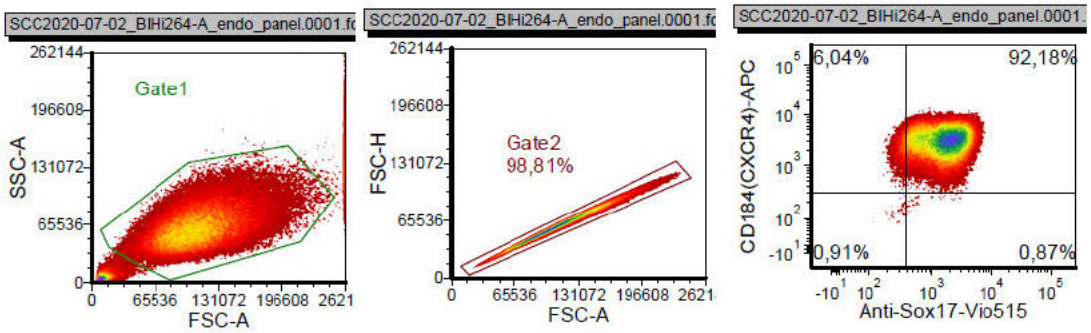


20200702 [redacted] Trilineage with BIHi264-A endoderm differentiation

negative control



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



Conclusion

The cell line BIHi264-A at passage 18 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.

Responsible person / date: [redacted] / 22.03.2021