Certificate of Analysis

CELL LINE NAME	**BIHi271-A**	hPSCreg Link: *https://hpscreg.eu/cell-line/BIHi271-A*
DONOR GENDER/AGE:	☐ Male ☒ Female ☐ unknown Age: n.a.	
TYPE OF DISEASE / GENETIC MODIFICATIONS	n.a.	
BANK	Master Bank, ID MB01 , Passage 20, Freezing Date: 23.10.2020	
FREEZING METHOD	Bambanker	
CULTURE PLATFORM	Feeder Independent	
	Medium: E8	Coating:
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		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	not done
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

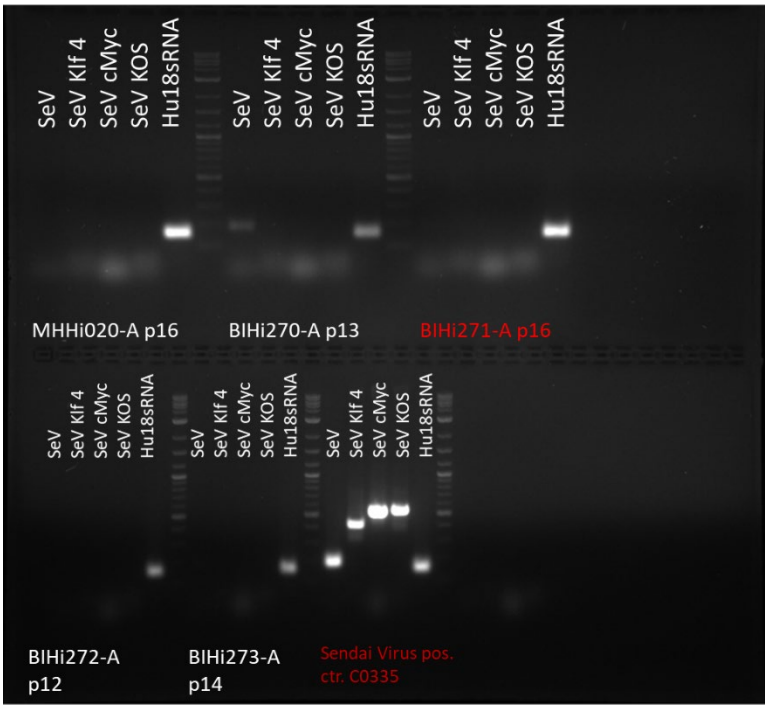
Date: 05.01.2023

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

Cell line name	BIHi271-A
Passage No.	16
Bank	MB01
Name operator	[redacted]
Date of testing	16.10.2020
Protocol	8.4. Testing for remaining Sendai virus_CytoTune 2.0

Results

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



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

PCR Results - Conclusion

The cell line BIHi271-A is tested negative for Sendai virus.

Responsible person / date: [redacted] / 16.10.2020

Stem Cell Core Facility

Single Nucleotide Polymorphism (SNP)- Karyotype

Sample (cell type, ID)	iPSC	BIHi271-A	
Passage No.	22		
Bank ID	MB01		
DNA sample ID	D0359		
Chip-ID and Position	205443530050, R10C01		
Date of testing	21.07.2021		
Call Rate	0.9922429		√
Gender (provided/estimated from chip data)	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

Genotype Analysis
GenomeStudio: GenomeStudio V2.0.5
Genotyping Module: V2.0.5

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.

Call Table

CNV regions found in BIHi271-A

Chr	Start	End	Size (bp)	CNV Value	Variant Type	Number of Genes*
2	96,741,795	98,994,641	2,252,846	2	LOH	
7	10,233,678	11,246,456	1,012,778	2	LOH	
16	34,428,972	34,724,788	295,816	3	Gain	
X	6,763,910	7,770,460	1,006,550	2	LOH	
X	100,773,013	101,857,535	1,084,522	2	LOH	
X	55,355,711	57,142,874	1,787,163	2	LOH	

*Number of genes in CNV/LOH regions given only for **reportable** calls (see Appendix for details on genes in reported regions).

Interpretation

No reportable genomic abnormalities were detected in the BIHi271-A iPSC line at the stated level of resolution. Information about genes in the non-reportable 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

Praxis für Humangenetik
Beratung & Diagnostik

Zertifiziert bis 12/2017

Fachärzte/Innen für Humangenetik
Prof. Dr. med. Gundula Kadgien*
Dr. med. Eun Kyung Suk
(*angestellte Ärzte/Ärztinnen)

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Charité – Universitätsmedizin Berlin
[redacted]
BCRT / Charité / BIH iPS-Cell Core Facility
Föhrer Straße 15
13353 Berlin

Zytogenetische Untersuchung von Zelllinien

Sehr geehrter Herr Kollege,

wir berichten über die Passage 22 der Zelllinie BIHi271-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 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 3.2.2021

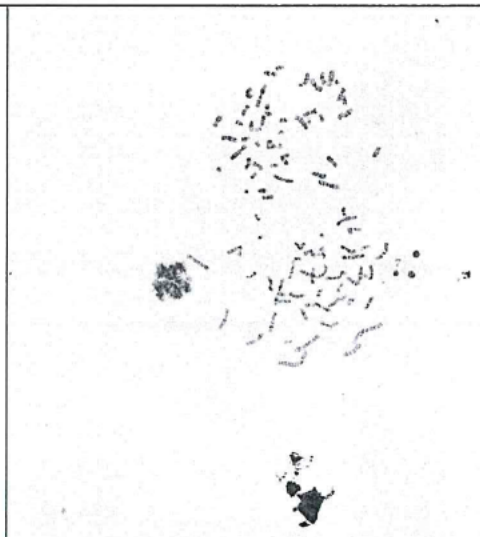
Mit kollegialen Grüßen

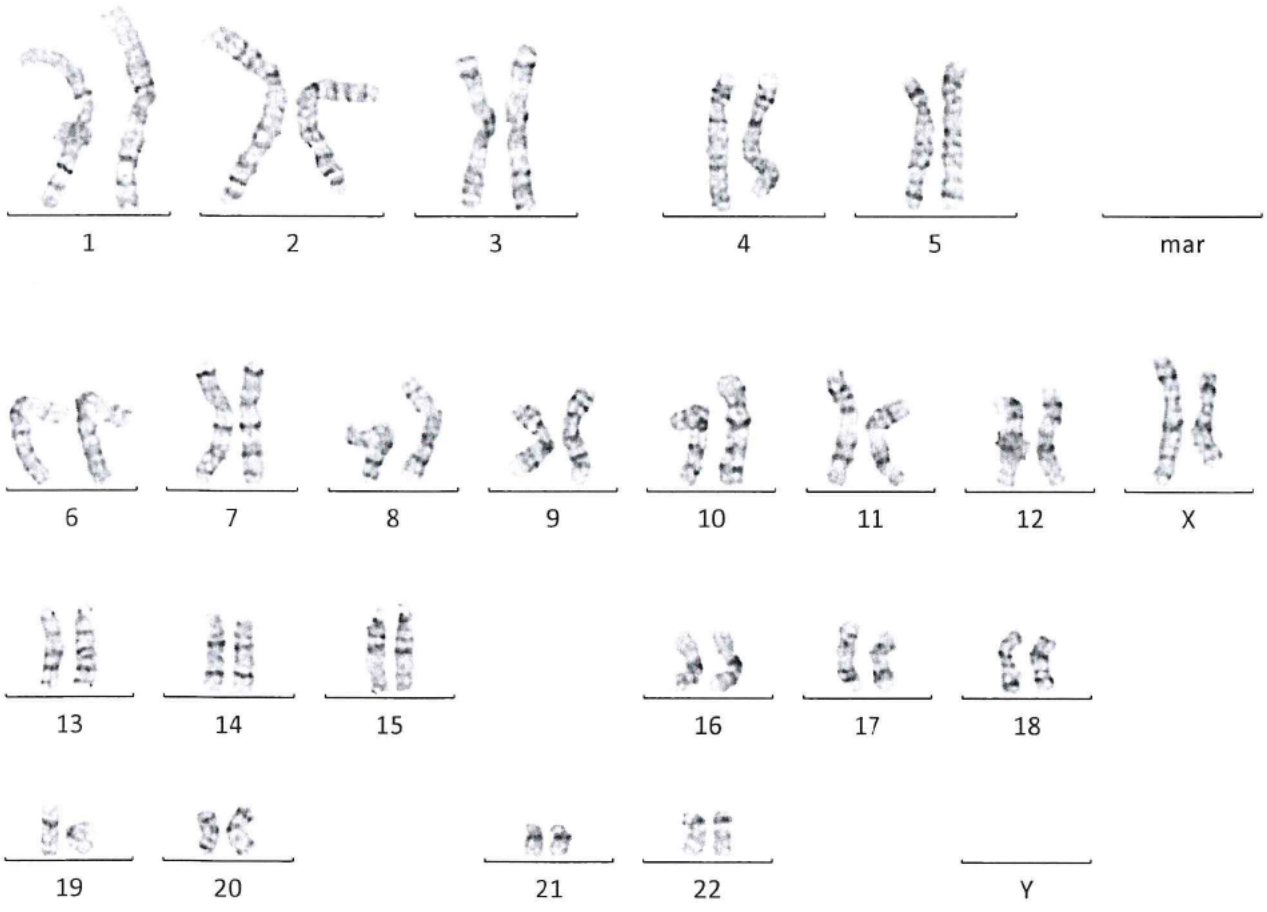
[redacted]

Praxis für Humangenetik

[redacted] - Fachärztin für Humangenetik

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



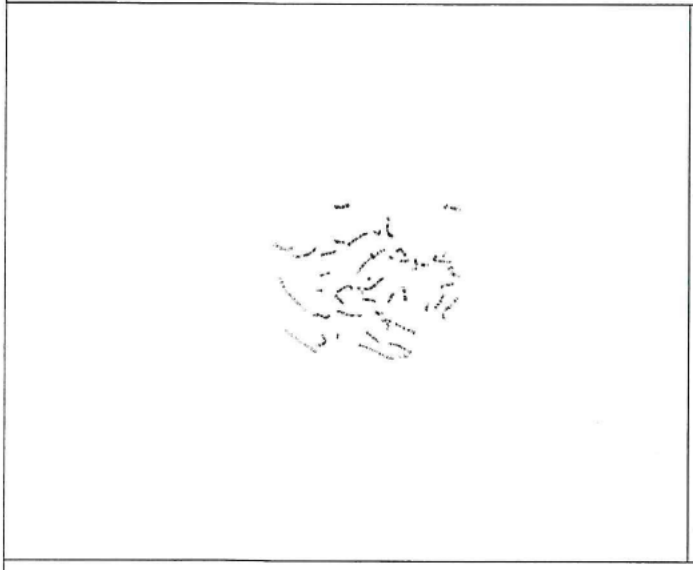


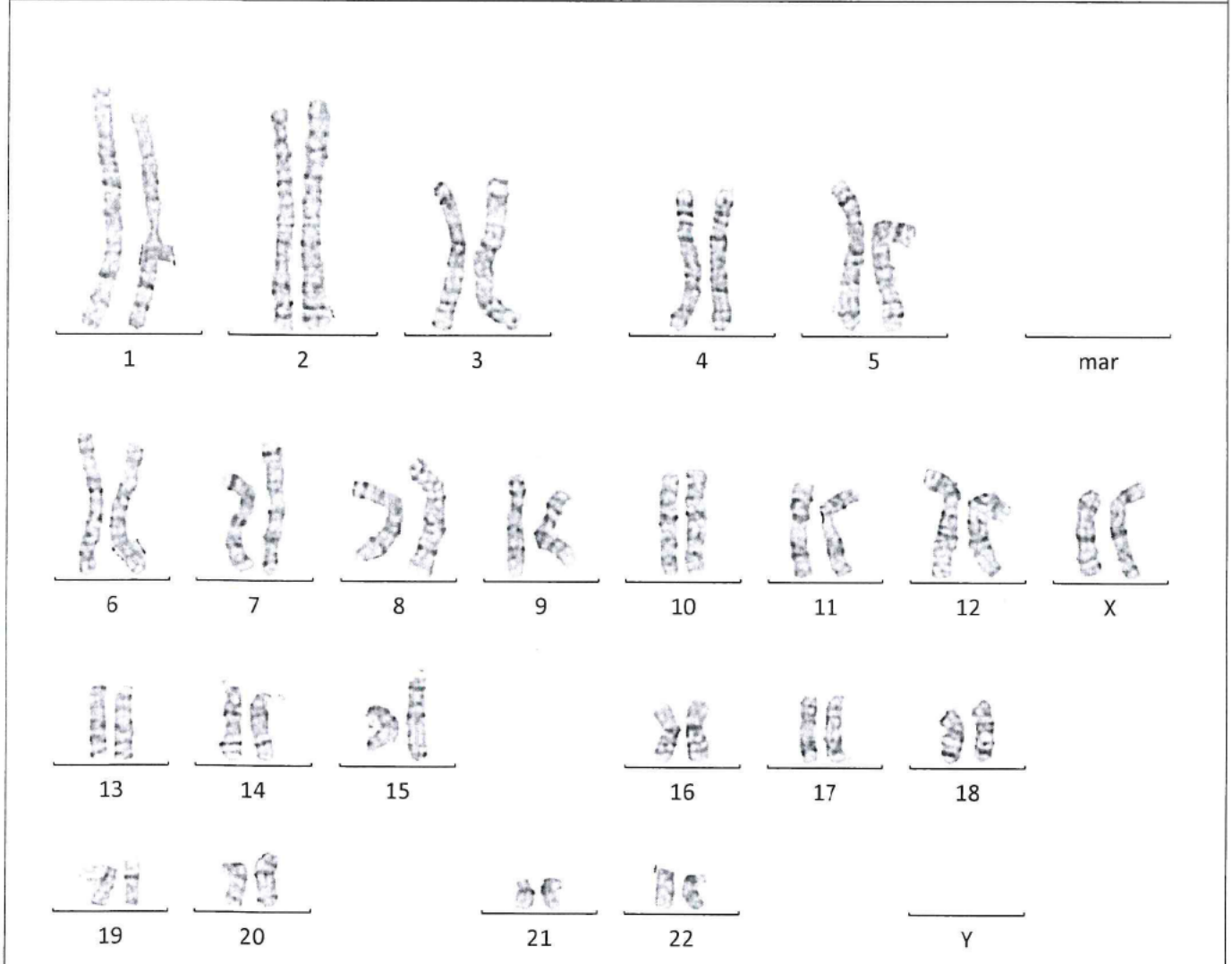
SZ BIHi271-A-MB01 • Cu p22 • Sl III • 030 46,XX

Praxis für Humangenetik

[redacted] - Fachärztin für Humangenetik

Friedrichstraße 147 - 10117 Berlin - Tel. [redacted]





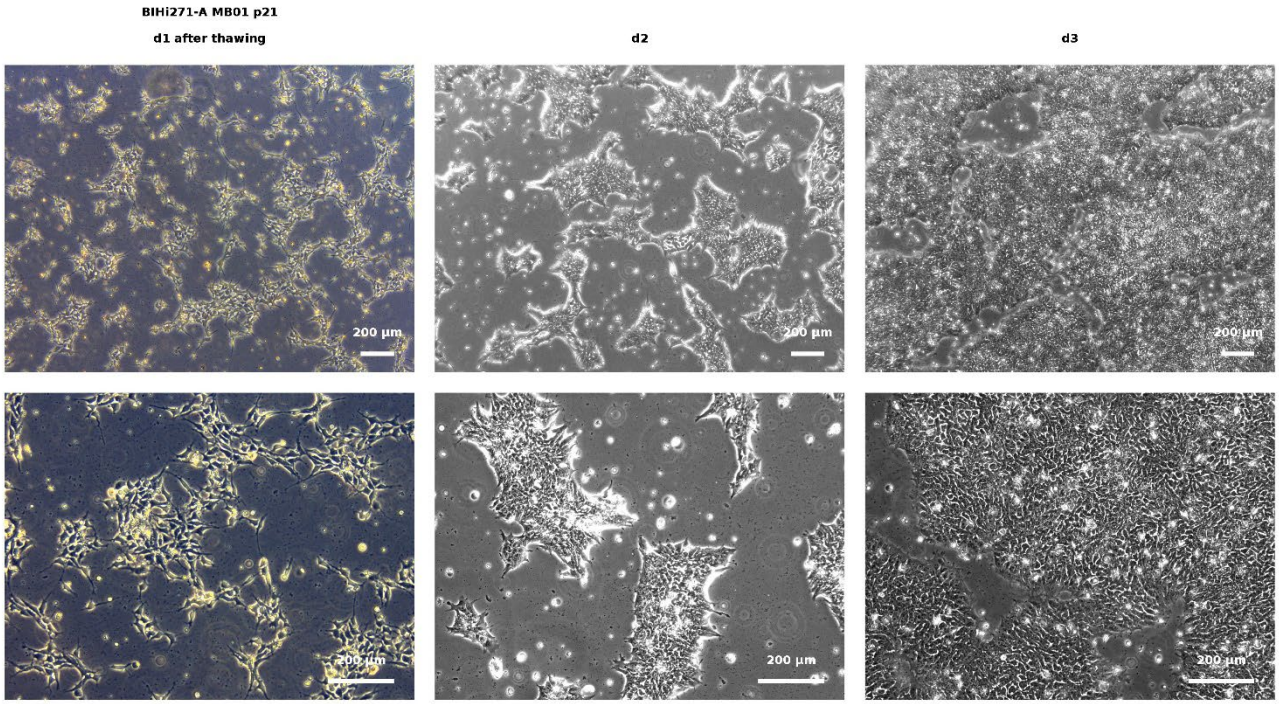
SZ BIHi271-A-MB01 • Cu p22 • SI III • 041 46,XX

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Viability after thawing**

Cell line name	BIHi271-A
Passage No.	P21
Bank	MB01
Name operator	[redacted]
Date of testing	20.11.2020

An aliquot of the master cell bank was thawed and monitored during antibiotics-free cultivation. ROCK Inhibitor was used only during the first 24 hours.

Images:



Conclusion:

The cell line BIHi271-A MB01 shows typically morphology of undifferentiated hPSC after 3 days.

Responsible person / date: [redacted] / 10.12.2020

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

Cell line / Passage No.	BIHi271-A / p21
Cell bank	MB01
Operator name	[redacted]
Test date	01.12.2020
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	28,309	Passed
2 (pos. control)	25,567	28,196	Passed
3	>45	28,337	**Negative**

Conclusion

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

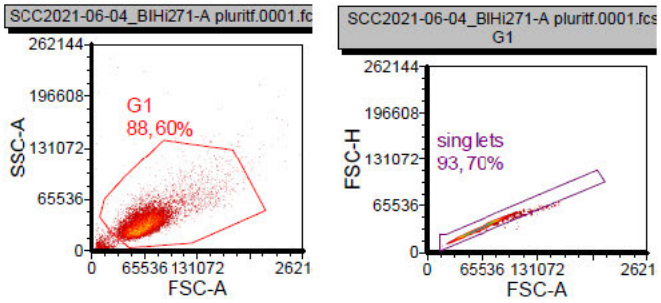
Responsible person / date: [redacted] / 09.12.2020

Stem Cell Core Unit

FACS analysis of markers in undifferentiated hPSCs

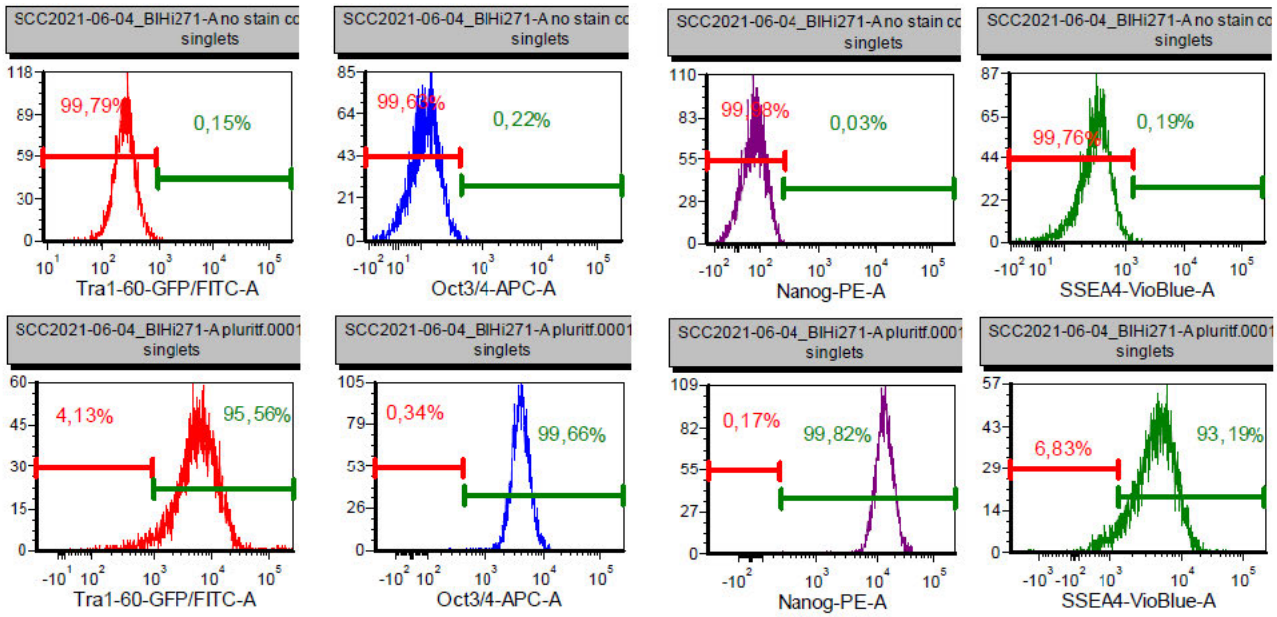
Cell line name	BIHi271-A
Bank ID	MB01
Passage No.	P22
Date of testing	04.06.2021
Protocol	7.14 FACS analysis of pluripotency markers

20210604_[redacted]_FACS analysis of markers of undifferentiated BIHi271-A MB01 p22



sample description	negative control (%)	sample (%)
Tra1-60-GFP/FITC-A	0,15	95,56
Oct3/4-APC-A	0,22	99,66
Nanog-PE-A	0,03	99,82
SSEA4-VioBlue-A	0,19	93,19

3 well of 6 WP: 1,52x10e6c, 88% viability
staining of 3x10e5c/tube



Conclusion

The cell line BIHi271-A at passage 22 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] / 07.06.2021

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

Validation of pluripotent differentiation potential

Cell line name	BIHi271-A
Passage No.	P22
Name operator	[redacted]
Date of testing	11.06.2021

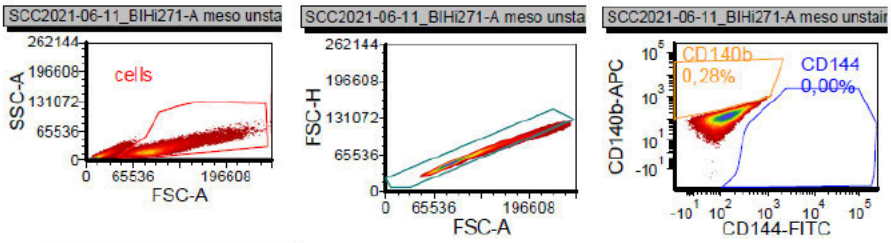
Method

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.

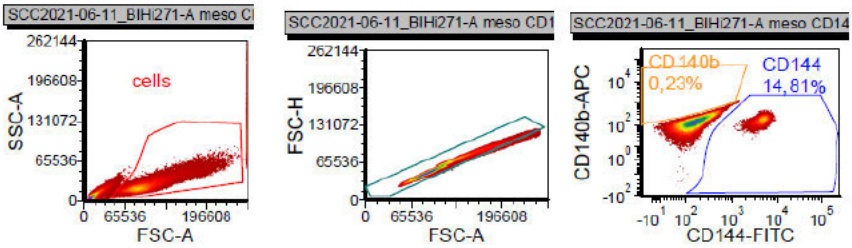
Result

20210611[redacted] Trilineage with BIHi271-A MB01 p22 mesoderm differentiation

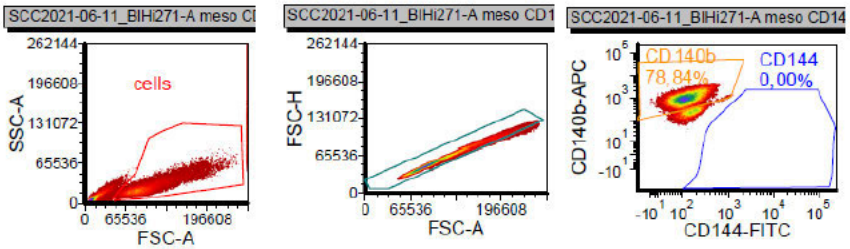
negative control



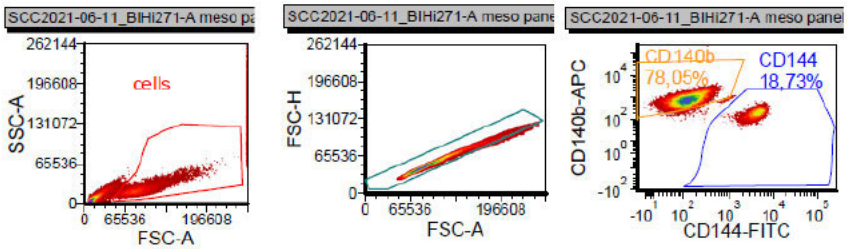
CD144-FITC only



CD140b-APC only



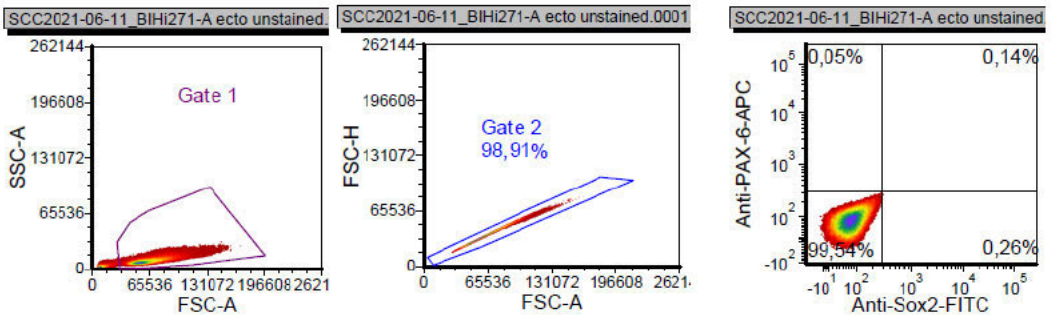
CD144-FITC
CD140b-APC



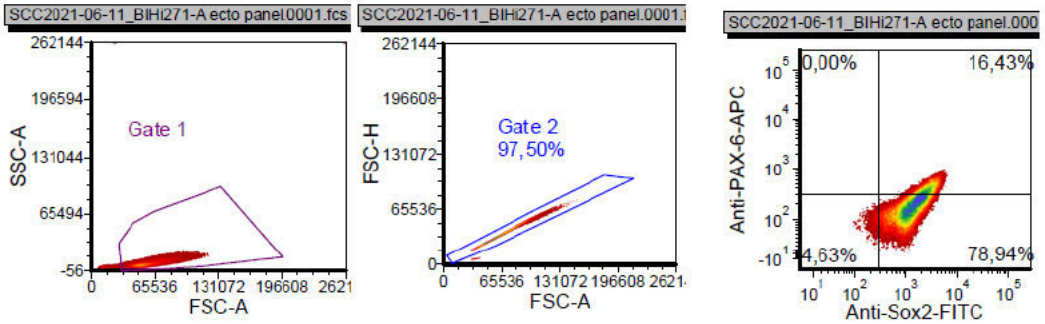
Validation of pluripotent differentiation potential

20210611 [redacted] Trilineage with BIHi271-A MB01 p22 ectoderm differentiation

negative control

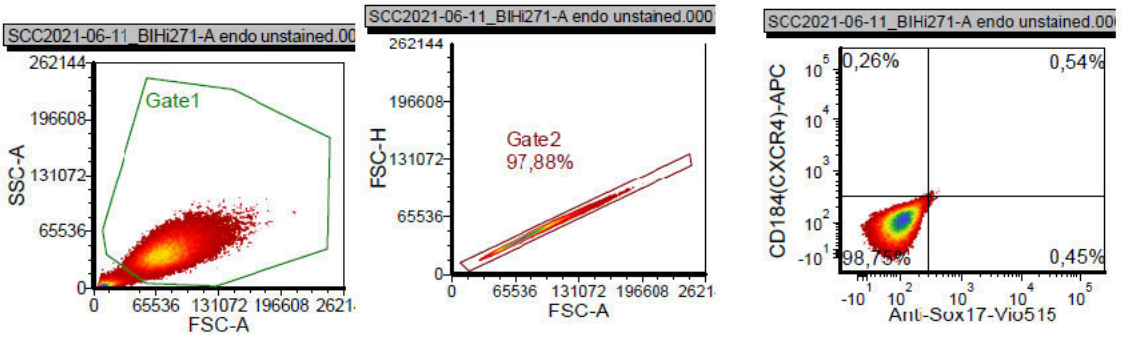


Anti-PAX-6-APC, human
Anti-Sox2-FITC, human

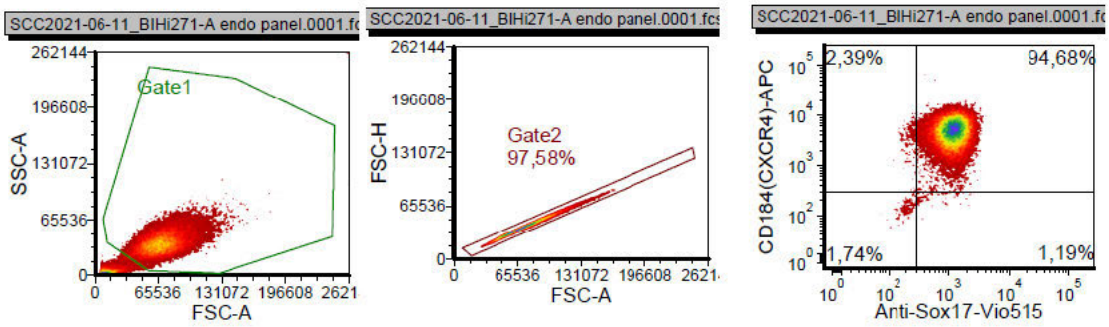


20210611 [redacted] Trilineage with BIHi271-A MB01 p22 endoderm differentiation

negative control



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



Conclusion

The cell line BIHi271-A at passage p22 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] / 11.06.2021

BIH Berlin Institute of Health @Charité	Stem Cell Core Unit **Cell Line Identity (STR Analysis)**

Cell line name	BIHi271-A
Bank ID	MB01
Passage No.	22
Date of testing	21.12.2021
Protocol	8.05. STR DNA Profiling Analysis

The GenePrint® 10 System (Promega Corporation) allows co-amplification and three-color detection of nine human loci, including the ASN-0002 loci (TH01, TPOX, vWA, Amelogenin, CSF1PO, D16S539, D7S820, D13S317 and D5S818) as well as D21S11. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92 × 109.

Results

	TH01		D21S11		D5S818		D13S317		D7S820		D16S539		CSF1PO		AMEL		vWA		TPOX	
BIHi271-A	8	10	31	32.2	12	13	11	12	10	12	11	12	9	10	X	X	17	19	8	10