

## Certificate of Analysis

CELL LINE NAME	<b>MDCi237-B</b>	hPSCreg Link: <a href="https://hpscereg.eu/user/cellline/edit/MDCi237-B">https://hpscereg.eu/user/cellline/edit/MDCi237-B</a>
DONOR GENDER/AGE:	<input checked="" type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> unknown Age: 1-4 Jahre	
DISEASE PHENOTYPE / GENETIC VARIANT		
BANK	MB01, ID 01, Passage 14, Freezing Date: 18.03.2022	
FREEZING METHOD	Single cells in Bambanker	
CULTURE PLATFORM	Feeder Independent	
	Medium: StemMACS	Coating: Geltrex
REPROGRAMMING	Sendai virus (CytoTune 2.0) Vector details (e.g. Kit, Pub, AddgeneNr):	
GENETIC MODIFICATION	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no Targeting Vector: <input type="checkbox"/> TALEN, <input type="checkbox"/> CRISPR, <input type="checkbox"/> ZNF, Addgene: <input type="checkbox"/> Isogenic control/SNP <input type="checkbox"/> Gene knock-out <input type="checkbox"/> Transgene knock-in	
	Parental/isogenic cell line	#11624 Fibroblasts
	Target gene/Transgene/Locus (het/hom?)	
	Validation (e.g. PCR, sequencing)	

TEST DESCRIPTION	Test Method	Test Specification	Result
STERILITY (viral pathogens)	<input type="checkbox"/> Blood screening Donor <input type="checkbox"/> PCR (primary cells) <input type="checkbox"/> PCR (iPS clone/subclone)	HBV, HCV, HIV negative	Pass
STERILITY (mycoplasma)	Test Method	No contamination detected	Pass
STERILITY (bacteria/ yeast/ fungi)	Culture for 7 days in antibiotic free medium	No contamination detected	Pass
REPROGRAMMING VECTORE CLEARANCE	<input checked="" type="checkbox"/> PCR <input type="checkbox"/> AB staining <input type="checkbox"/> Confirmed in parental line	Vector not present	Pass
VIABILITY / MORPHOLOGY	Phase contrast microscopy of cells at 24, 48, and 72 hrs	Growth rate and confluency typical of hPSCs	Pass
UNDIFFERENTIATED PHENOTYPE	Markers for undifferentiated hPSCs <input type="checkbox"/> IF-Staining <input checked="" type="checkbox"/> FACS <input type="checkbox"/> other	Expression of at least three pluripotency markers detected	Pass
	<input type="checkbox"/> Pluritest	Pluripotency and Novelty Scores above threshold	not done
PLURIPOTENT DIFFERENTIATION POTENTIAL	3-germ layer differentiation: <input type="checkbox"/> spontaneous (e.g. EB formation)	Detection of markers for cells from the three germ layers	not done
	<input type="checkbox"/> directed differentiation	Successful differentiation to cells of all three germ layers	not done
	<input type="checkbox"/> Teratoma formation	Observation of tissues derived from the three germ layers	not done

## Certificate of Analysis

KARYOTYPE	PerkinElmer KaryoLite BoBs™	Karyotype matches Donor	not done
	Virtual karyotyping using Illumina OMNI-EXPRESS-8v1.6 Chip	No significant changes compared to the primary cells detected	Pass
	G-Banding	Karyotype matches Donor	not done
IDENTITY (STR ANALYSIS)	Promega GenePrint® 10 System	Identical to profile of primary cells	Pass

Date: 11.05.2022

signature:

/Carolin Genehr

<b>Cell line name</b>	11624 Fibroblast, <b>MDCi237-B</b>
<b>Gender</b>	Male
<b>Passage No.</b>	5, 15
<b>Name operator</b>	Sebastian Diecke, Gabi Born
<b>Date of testing</b>	19.04.2022

**Specifications:**

iPSCs were karyotyped using the ISCAN machine and the Illumina platform OMNI-EXPRESS-8v1.6 Chip (Marker coverage 958,497 spanning whole human genome). The analysis was performed by using Karyostudio 1.3 software based on the information of GRCh36/hg18 dataset.

The analysis software stringency settings used to identify aberrant regions are listed below. Reportable copy number changes are gains and losses greater than 0,4 Mb and regions of LOH (loss of heterozygosity) above 3 Mb (in accordance with WiCell criteria (service provider pluripotent stem cell banking and characterization).

In Known Regions	Type of CNV	Size Threshold	Markers Threshold	CNV Confidence Threshold
Inside	Gain	100000	15	100
Inside	Loss	75000	15	100
Inside	CNLOH	3000000	30	100
Outside	Gain	200000	15	100
Outside	Loss	150000	15	100
Outside	CNLOH	8000000	30	100

This method can detect the following aberrations:

- Genomic gains and losses
  - Copy number variants (CNVs)
  - Duplications/deletions
  - Unbalanced translocations
  - Aneuploidies
- Copy neutral aberrations Loss of heterozygosity (LOH) / Absence of heterozygosity (AOH)
- >20% mosaicism (for example: cultures where >1 of 5 cells is trisomy 12)

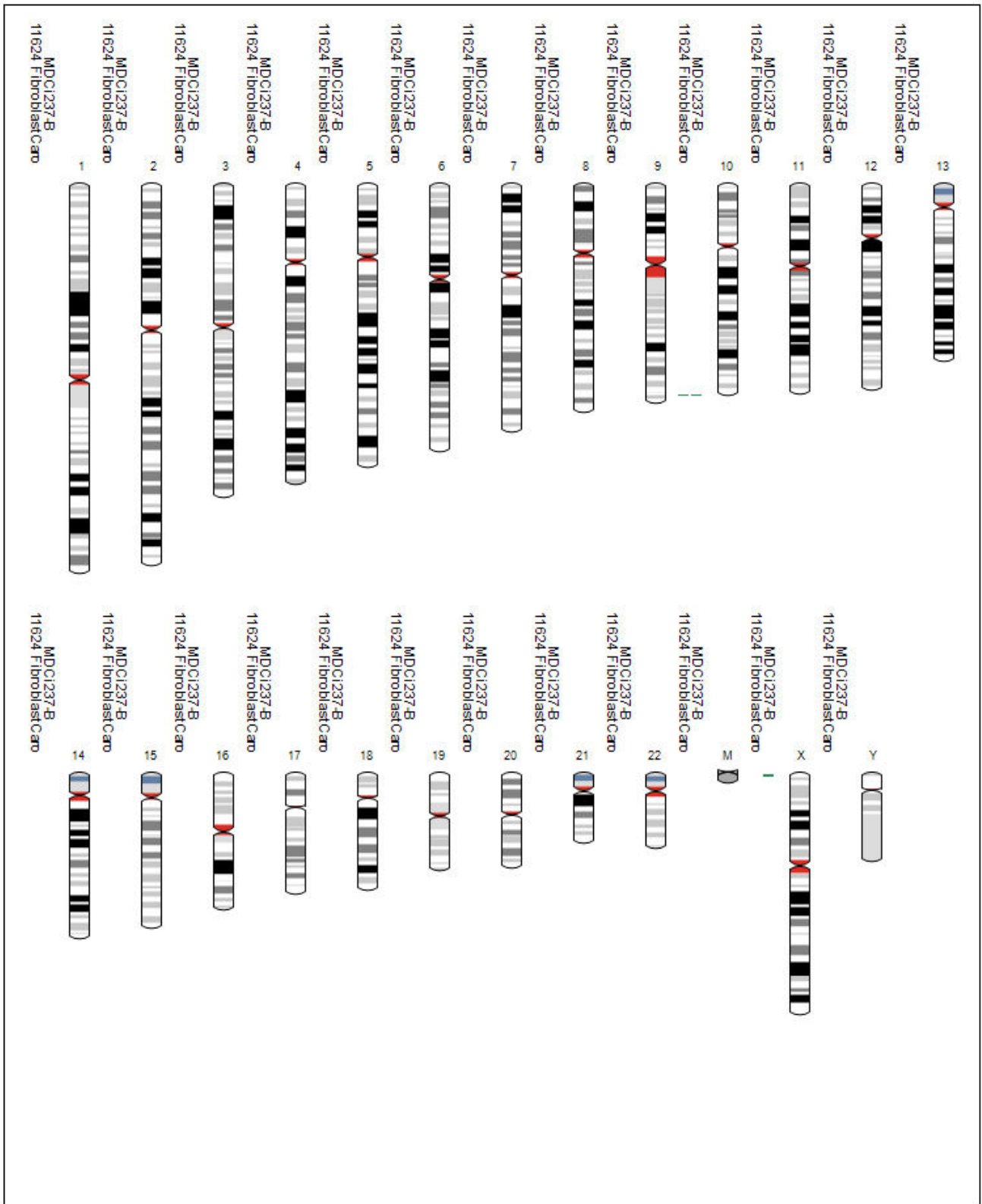
Limitations:

Other aberrations like the once listed below can't be detected using this array.

- Balanced translocations
  - Robertsonian
- Balanced insertions
- Inversions
- <20% culture mosaicism (for example: cultures where 1 of 5 cells is trisomy 12)
- Chromosomal position of genomic gains

**Virtual Karyotype:**

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



**Results:**

Estimate of the physical copy number of a detected region:

- 0 indicates a homozygous deletion (loss of both copies)
- 1 indicates a hemizygous deletion (loss of one copy)
- 2 indicates a copy-neutral loss of heterozygosity (e.g., Uniparental disomy (UPD or autozygosity)
- 3 indicates a duplication (gain of one copy)
- 4 indicates a copy number of 4 or above

Sample ID	Chr	Start	Stop	Length	Value
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**Interpretations:**

- The cell line MDCi237-B has an inconspicuous karyotype.
- There were some reportable copy number changes (CNV's) within the tested clone but below the threshold values mentioned above.
  - Refer to the data section and the excel table "table of affected genes" and see above
- Besides the information listed in the cytogenetic report about known diseases linked to the reported aberrations the UCSC Genome Browser (<https://genome.ucsc.edu>) and Decipher (<https://decipher.sanger.ac.uk/search>) may provide additional information on the detected regions.

**Sebastian Diecke** Digitally signed by Sebastian Diecke  
Date: 2022.05.17 08:49:43 +02'00'

Responsible person / date: Sebastian Diecke/ 26/04/2022

**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>

**Attachments:**

- Cytogenetics Report
- Table of affected genes
- Karyogram only

<b>Cell line name / Passage No.</b>	MDCi237-A, <b>MDCi237-B</b> , 11624
<b>Bank</b>	MB01, <b>MB01</b> ,Fibros
<b>Name operator</b>	Gabi Born
<b>Date of testing</b>	24.05.2022
<b>Protocol</b>	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  $2.92 \times 10^9$ .

Date	Passage	Cell line	TH01		D21S11		D5S818		D13S317		D7S820		D16S539		CSF1PO		AMEL		vWA		TPOX	
			6	7	29	29	11	13	11	12	9	10	11	12	10	11	X	Y	16	18	8	9
24.05.22	14	MDCi237-A	6	7	29	29	11	13	11	12	9	10	11	12	10	11	X	Y	16	18	8	9
<b>24.05.22</b>	<b>15</b>	<b>MDCi237-B</b>	<b>6</b>	<b>7</b>	<b>29</b>	<b>29</b>	<b>11</b>	<b>13</b>	<b>11</b>	<b>12</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>10</b>	<b>11</b>	<b>X</b>	<b>Y</b>	<b>16</b>	<b>18</b>	<b>8</b>	<b>9</b>
24.05.22	5	11624	6	7	29	29	11	13	11	12	9	10	11	12	10	11	X	Y	16	18	8	9

### Results

The Alleles of the cell lines MDCi237-A, MDCi237-B and the 11624 the 10 STR Loci are identically.

### Conclusion

All samples tested are from the same donor.

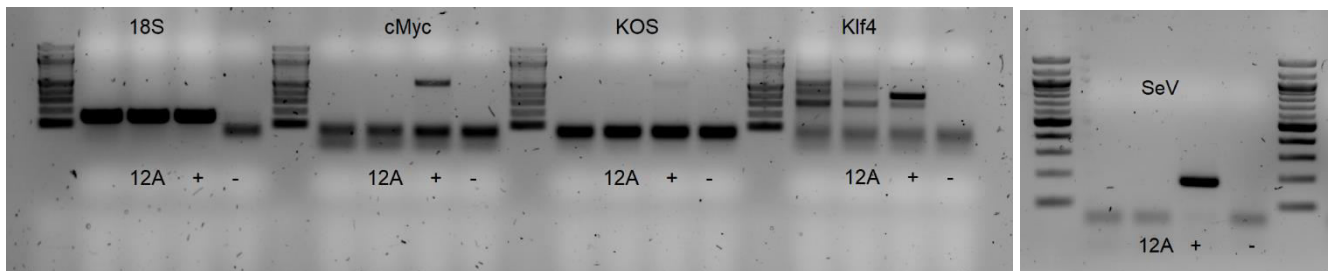
Responsible person / date: Gabi Born / 25.05.2022

<b>Cell line name</b>	MDCi237-B
<b>Passage No.</b>	10
<b>Name operator</b>	Carolin Genehr
<b>Date of testing</b>	15.03.2021
<b>Protocol</b>	8.4. Testing for remaining Sendai virus_CytoTune 2.0
<b>Sample</b>	12A: MDCi237-B +: positive control -: water

**Results**

2 % standard agarose gel with DNA stain RotiSafe 5µL/100 mL

PCR picture:



Primer :  
1.Hu18SRNA 2.cMyc 3.KOS 4.Klf4 5.SeV

**PCR Results - Conclusion**

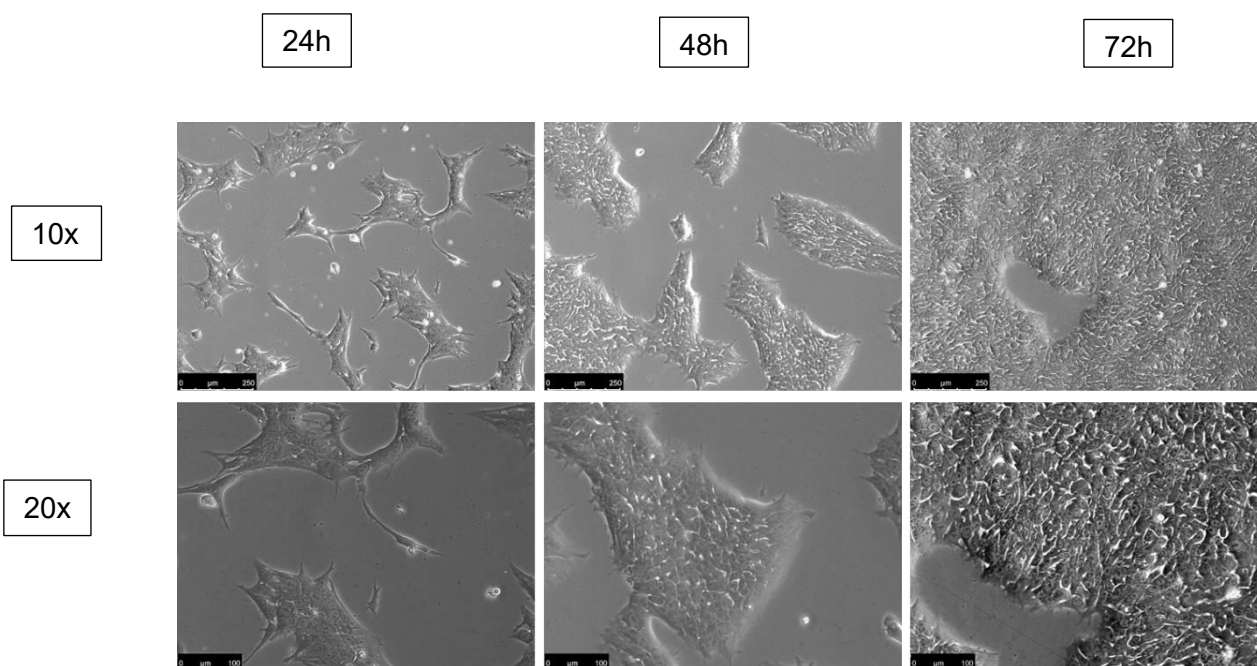
The cell line MDCi237-B is tested negative for Sendai virus.

Responsible person / date: Carolin Genehr / 17.03.2022

<b>Cell line name</b>	MDCi237-B
<b>Passage No.</b>	15
<b>Bank</b>	Master bank 01
<b>Name operator</b>	Carolin Genehr
<b>Date of testing</b>	06.-08.04.2022

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:**

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

The cell line MDCi237-B MB01 shows typical morphology of undifferentiated hiPSC.

Responsible person: Carolin Genehr / date: 25.04.2022

<b>Cell line / Passage No.</b>	MDCi237-B / p17
<b>Cell bank</b>	MB01
<b>Operator name</b>	Norman Krüger
<b>Test date</b>	05.05.2022
<b>Protocol</b>	8.1.3 Mycoplasma testing_qPCR Minerva
<b>Samples</b>	1: Negative Control (culture medium of Cell Line tested) 2: Positive Control (Mycoplasma DNA from <i>Venor®GeM qOneStep Kit</i> ) 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.

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

**Results**

<b>Sample</b>	<b>Ct of Mycoplasma DNA</b>	<b>Ct of Internal amplification DNA</b>	<b>Result</b>
1 (neg. control)	>45	28,1	Passed
2 (pos. control)	25,6	28,3	Passed
3	>45	28,2	<b>Negative</b>

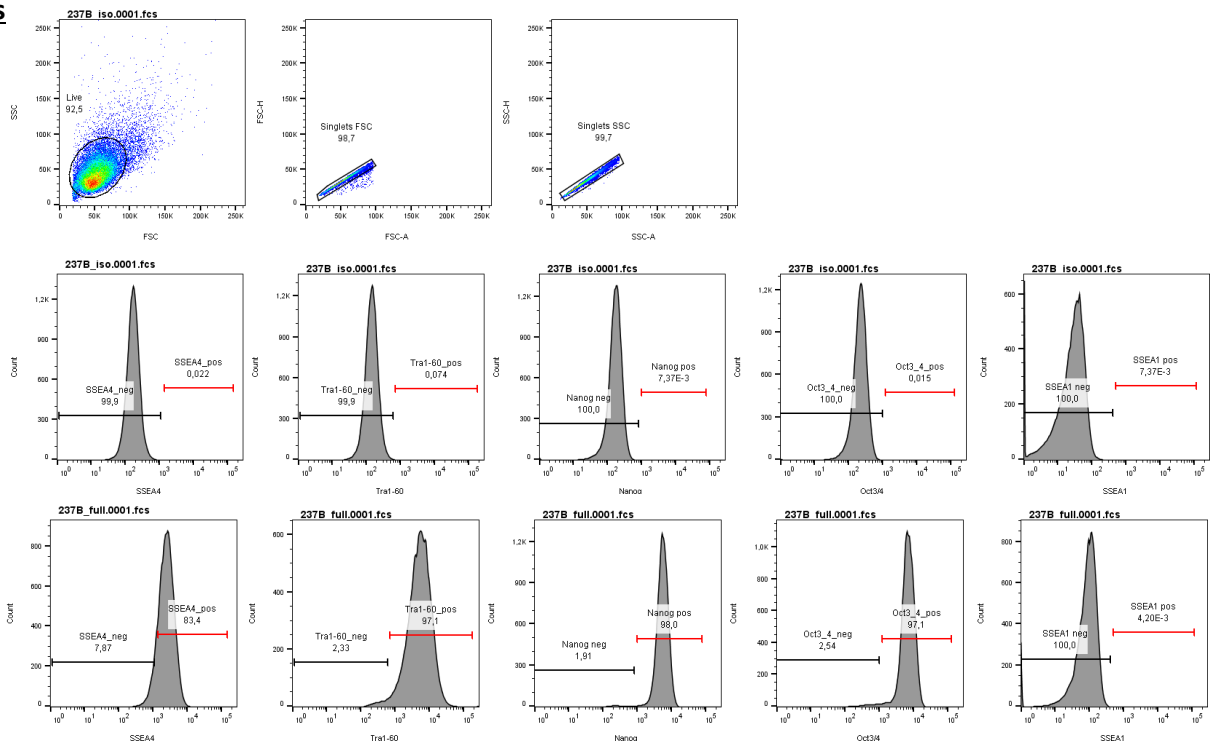
**Conclusion**

The cell line MDCi237-B MB01 p17 was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Responsible person / date: Norman Krüger / 05.05.2022

<b>Cell line name</b>	MDCi237-B
<b>Passage No.</b>	16
<b>Bank</b>	Masterbank 01
<b>Name operator</b>	Sandra Schommer
<b>Date of testing</b>	13.04.2022
<b>Protocol</b>	7.14 FACS analysis of pluripotency markers

**Results**



	SSEA4	Tra1-60	Nanog	Oct3/4	SSEA1
<b>isotype</b>	<b>0,02%</b>	<b>0,07%</b>	<b>0%</b>	<b>0,02%</b>	<b>0%</b>
<b>positive</b>	<b>83,4%</b>	<b>97,1%</b>	<b>98,0%</b>	<b>97,1%</b>	<b>0%</b>

**Conclusion**

The Masterbank of cell line MDCi237-B at p16 shows positive FACS results (over 80% positive) for the tested undifferentiated stem cell markers Tra1-60, OCT3/4, NANOG and SSEA-4. Additionally it shows negative FACS results (lower than 20% positive) for the differentiation marker SSEA-1.

Responsible person Sandra Schommer / date: 19.04.2022