Sterility (Mycoplasma, Bacteria/Yeast/Fungi)

Cell line / Passage No.	Orang iPSCs Dokana cl. 8 scl. H / P17
Cell bank	MB01
Operator name	Jeong-Eun Lee
Test date	29.03.2023
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	27.52	Passed
2 (pos. control)	24.18	27.36	Passed
3	>45	27.34	**Negative**

Conclusion

The cell line Orangutan iPSC Dokana cl. 8 scl. H MB01 P17 was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Responsible person / date: Jeong-Eun Lee/ 29.03.2023