

Characterization of hPSC

| Cell Line Name | hiPSC-TLR8KO-A10 | | |
|--|---|-----------|-----------------|
| Alternative name | CMC-hiPSC-003(TLR8KO-A10) | | |
| Type of Cell Line | hiPSC | | |
| Depositor (Institution) | Korea National Institute of Health | | |
| Passage # | p39* | | |
| Day of Cell Freezing | 20200627* | | |
| Analysis | Result | Passage # | Day of analysis |
| Cell viability | Pass(80.2±0.9%) | p39 | 20200916 |
| Authentication (STR) | Pass | p39 | 20210608 |
| Mycoplasma test (PCR) | Pass | p39 | 20200922 |
| Cell attachment and colony morphology | Pass | p39 | 20211001 |
| Bacterial, and fungal contamination test | Pass | p39 | 20200908 |
| Viral contamination test | Pass | p39 | 20200904 |
| Karyotype (G-banding) | 46,XY | p39 | 20200915 |
| Stem Cell Marker Expression | | | |
| · AP staining | Pass (positive) | p31 | 20181026 |
| · ICC | Pass (positive) | p31 | 20180719 |
| · qRT-PCR | Pass (positive) | p31 | 20181109 |
| Differentiation Marker Expression | | | |
| · EB fomatation | Pass (EB14d) | p31 | 20181109 |
| · qRT-PCR | Pass (positive) | p31 | 20181109 |
| · Hematopoietic/ macrophage lineage | Pass (positive) | p36 | 20190528 |

* Freezing media : Stem-cellbanker (Zenoaq #BLC-3-1)

Cell Culture Condition

- Feeder(matrix) : - Vitronectin (Gibco, A14700)
- Media : - Essential 8 (Gibco, ThermoFisher, A1517001)
- Clone R (Stem Cell Technol., CAT#05888) or Y27632, at thawing
- Passage (Cell dissociation) - EDTA or Gentle Cell Dissociation Reagent (Stem cell Technol, 07174)
- EZPassage (Thermo-Fisher 23181010)

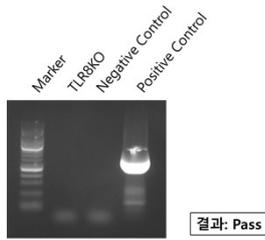
Genetic Modification

- Parental Cell - CMC-hiPSC-003(Catholic University of Korea)
- Genetic modification - CRISPR/Cas9, TLR8 knock-out

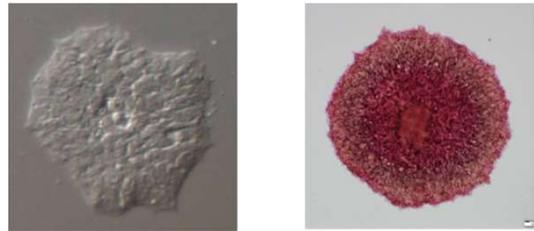
Reference

Han HJ et al., Generation of a KSCBi005-A-5(TLR8KO-A10) homozygous knockout human induced pluripotent stem cell line using CRISPR/Cas9., Stem Cell Res. 2019 Oct;40:101561. doi: 10.1016/j.scr.2019.101561.

Mycoplasma contamination test

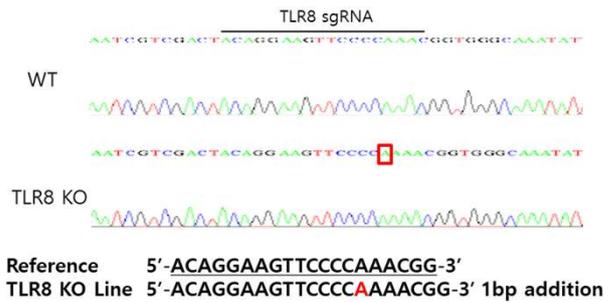


Cell Morphology AP staining



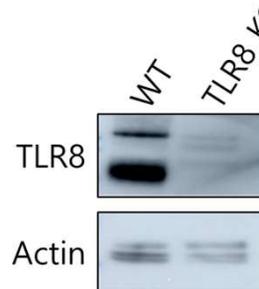
Microbial contamination test

TLR3 Knock-out (Sequencing)



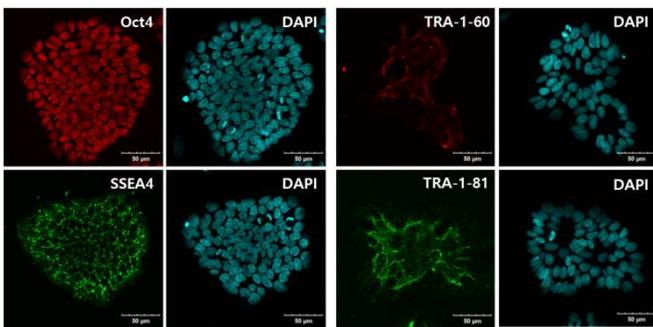
Karyotype

TLR8 Expression in hematopoietic cells (macrophage)



Stem cell marker gene expression

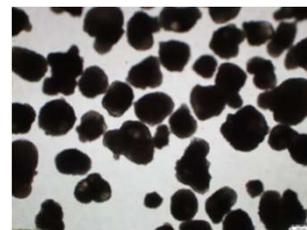
<ICC>>



Differentiation

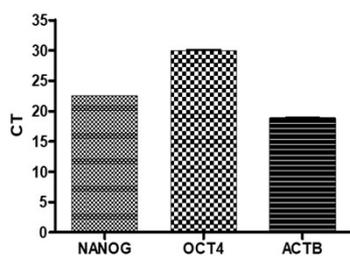
<EB formation>

EB 14 day



<qRT-PCR>

| 유전자 | 결과 (CT mean) |
|-------|--------------|
| NANOG | 22.53 |
| OCT4 | 29.97 |
| ACTB | 18.88 |



<qRT-PCR>

| 유전자 | 결과 (CT mean) |
|-------|--------------|
| ACTB | 18.25 |
| PAX6 | 25.80 |
| SOX17 | 23.72 |
| AFP | 31.17 |
| T | 28.25 |
| HAND1 | 21.31 |

