

NAME

UAB-iPSC-PKHD1KO#1

Unique Identifier

UABiPGP1-E3C11 PKHD1 C93_111del/99^100insA

Provider

UAB - PKD Center

General Information

Organism

Homo sapiens (human)

if mouse; strain information

NA

Tissue

skin

Cell Type

iPSC (parental line PGP1 iPSC)

Product Format

Frozen 1.0 mL

Culture Properties

Adherent

Biosafety Level

Age

53 year old

Gender

Male

Applications

These cells are proposed to be a valuable cell model for studying PKD or other renal associated diseases

Storage Conditions

Liquid nitrogen

Characterteristics

Morphology on plastic

2D growth - compact colonies

Growth in 3D culture

ND

Polycystin expression

ND

Culture Method

Complete Growth Medium

mTeSR™ Plus (STEMCELL # 100-0276) Do not thaw in a 37°C water bath. Warm to room temperature. Plates were coated with Geltrex™ LDEV-Free Basement Membrane Matrix (ThermoFisher; A1413302)

Subculturing Protocol

Remove and discard culture medium. Rinse cells with Phosphate Buffered Saline, (PBS). Add ACCUTASE™ Cell detachment solution (STEMCELL; # 07920); observe cells under microscope until cell layer is lifted (usually 3-5 minutes). Complete media is then added to neutralize. Transfer cells and centrifuge to obtain pellet. Resuspend cells in complete growth medium and aliquot to fresh culture flasks/dishes coated with Matrix. Split ratio 1:4 – 1:6. Media should be replaced daily or every other day. Maintain cultures at 37°C, 5% CO₂, in humidified incubator

Cryopreservation and thaw

Freeze media: CryoStor® CS10 (STEMCELL; #7931)

After thawing cell, iPSC can be cultured first in mTeSR plus with CloneR™2 supplement (STEMCELL; # 100-0691) in coated plate for better survival. Regular complete media can be replaced one day later.

Specifications

guide used for targeting

TGATCCACGTTCCCCCTGCA AGG

DNA Sequencing

PKHD1 gRNA

WT sequence CTGAAGAAAGGTAGCCTTGCA GGGGGAACGTGGATCACAGTCATTTTTGAT

CRISPR allele 1 CTGAAGAAAGGTAG-----ATCACAGTCATTTTTGAT

CRISPR allele 2 CTGAAGAAAGGTAGCCTTGCAAGGGGGAACGTGGATCACAGTCATTTTTGAT

genotyping

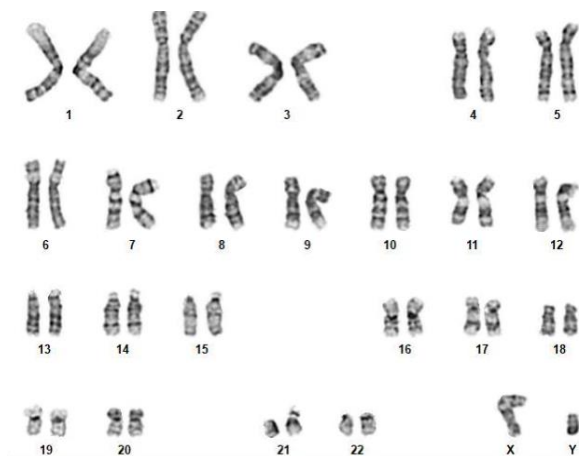
PCR conditions: Forward: 5'-GGGCTTCTGATGATGTGTTTCC-3'
Reverse: 5'-GTGCTAAGATCCTGGGGGTAA-3'
size 605bp; Taq DNA Polymerase (TaKaRa;R001A)
98 °C 30 sec; {98 °C 10 sec, 60 °C 10 sec, 72 °C 30 sec, 35 cycles}, 72 °C 5min--4 °C
PCR samples were running on 1% agarose gel and purified for Sanger sequencing. PCR product were further cloned into TOPO™ vector to validate CRISPR mutations on each allele.

DNA Finger printing

DNA Profile:
FGA 22, 22
TPOX 8, 9
D8S1179 13, 13
vWA 18, 18
Penta_D 8, 14
CSF1PO 10, 11
D16SFGA 22, 22
TPOX 8, 9
D8S1179 13, 13
vWA 18, 18
Penta_D 8, 14
CSF1PO 10, 11
D16S539 11, 12
D7S820 10, 13
D13S317 8, 11
D5S818 9, 11
Penta_E 17, 17
D18S51 15, 16
D21S11 28, 30
TH01 7, 8
D3S1358 16, 19
Amelogenin X, Y

Karyotype: karyotype image (if available)

46,XY



Western:

ND

Reference

for cell culture

[https://cdn.stemcell.com/media/files/manual/10000007757-Maintenance of Human Pluripotent Stem Cells in mTeSR Plus.pdf? ga=2.189989227.1309618205.1674486214-2115379575.1666018991& gac=1.190206297.1671124978.Cj0KCQiAqOucBhDrARIsAPCQL1avl8sNqVSTw3XD22dxYYb7uVT6GyOPtHOH5l6P7u6S47m8QmvTVtAaArgWEALw_wcB](https://cdn.stemcell.com/media/files/manual/10000007757-Maintenance%20of%20Human%20Pluripotent%20Stem%20Cells%20in%20mTeSR%20Plus.pdf?ga=2.189989227.1309618205.1674486214-2115379575.1666018991&gac=1.190206297.1671124978.Cj0KCQiAqOucBhDrARIsAPCQL1avl8sNqVSTw3XD22dxYYb7uVT6GyOPtHOH5l6P7u6S47m8QmvTVtAaArgWEALw_wcB)