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Loss of p57^{KIP2} expression confers resistance to contact inhibition in human androgenetic trophoblast stem cells.

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<Culture of hTSCs>

> Materials

Reagent	Supplier	Cat#
DMEM/F12	Wako	048-29785
30% BSA	Wako	017-22231
ITS-X	Wako	094-06761
L-Ascorbic acid	Wako	013-12061
VPA	Wako	227-01071
Penicillin-Streptomycin	Thermo Fisher Scientific	15140122
PBS	Wako	166-23555
TrypLE	Thermo Fisher Scientific	12604-021
EGF	Wako	053-07871
A83-01	Wako	035-24113
CHIR99021	Wako	034-23103
Y27632	Wako	036-24023
KSR	Thermo Fisher Scientific	10828028
iMatrix511	Wako	385-07361
Cell Banker 1	Nippon Zenyaku Kogyo	CB011
Nunc 6well plate	Nunc	140675

> Media preparation

▫ 10 mM A83-01

A83-01	10 mg
DMSO	2.37 ml

Store at -20°C

▫ 4 mM CHIR99021

CHIR99021	5 mg
DMSO	2.69 ml

Store at -20°C

▫ 10 mM Y27632

Y27632	5 mg
Sterile H ₂ O	1.48 ml

Store at -20°C

▫ 200 mM L-Ascorbic acid

L-Ascorbic acid	0.58 g
H ₂ O	10 ml

Filtration, Store at -20°C

▫ 100 µg/ml EGF

EGF	500 µg
PBS/0.2% BSA	5 ml

Store at -20°C

▫ TS basal medium: 500 ml

DMEM/F12	485 ml
BSA	2.5 ml
Penicillin-Streptomycin	2.5 ml
ITS-X	5 ml
KSR	5 ml
200 mM L-Ascorbic acid	0.5 ml

Store at 4°C, Use within one month

▫ TS medium: 40 ml

TS basal medium	40 ml
10 mM Y27632	10 µl
100 µg/ml EGF	10 µl
VPA	5 µl
10 mM A83-01	20 µl
4 mM CHIR99021	20 µl

Store at 4°C, Use within two weeks

➤ **Methods @ 6well dish**

1. Add 2 ml TS medium and 1 μ l iMatrix511 to each well
2. Keep the dish at 37°C for at least 10 minutes
3. Suspend $\sim 0.5 \times 10^5$ cells in the TS medium
4. Passage the cells every 3 days (Cells can be cryopreserved in Cell Banker 1)

➤ **Passaging cells**

1. Aspirate TS medium
2. Add 0.5 ml PBS and 0.5 ml TrypLE
3. Keep the dish at 37°C for ~ 15 minutes
4. Detach the cells by pipetting
5. Transfer the detached cells to a new tube containing 1 ml TS basal medium
6. Centrifuge at 380 g for one minute
7. Transfer the cell pellet into new wells (split ratio = 1:10 to 1:15)