NAME

Pax8rtTA;TetO-Cre;Pkd2$^{cond/cond}$ Immortalized clone #125

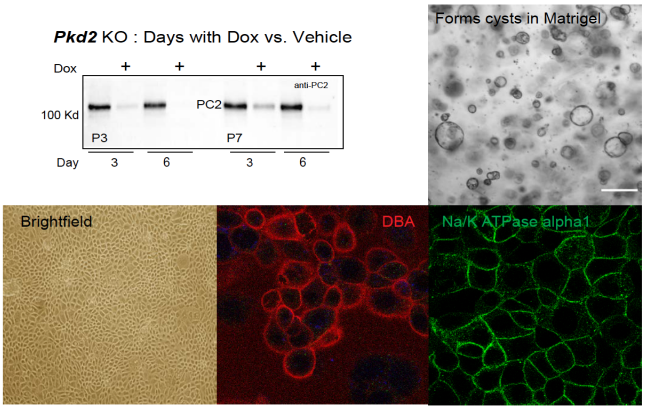
Unique Identifier	**MDCCEC 125**
Provider	Maryland PKD Center Cell Culture and Engineering Core (CCEC)

General Information

Organism	Mus musculus
if mouse; strain information	mixed
Tissue	Renal medulla, epithelium
Cell Type	Epithelial cells lines generated from Pax8 Tet-o-CrePkd2 fl/fl mice crossed with the immorto-mouse expressing the temperature sensitive SV40 large T-antigen.
Product Format	frozen 1.0 mL
Morphology	inner medullary Epithelial-like
Culture Properties	Adherent
Biosafety Level	NA
Age	Adult
Gender	male
Applications	These cells are proposed to be a valuable model system for understand the role of PKD2 and its gene product PC2 in the biology of renal epithelial cells
Storage Conditions	Liquid nitrogen vapor phase

Characteristics

Figures



Epithelial monolayer; TER = 2000 ohms*cm^2

Inner medullary kidney gene expression profile

Figure legend	This clone was derived from the renal medulla of male mouse, and the inner medullar characteristics have been confirmed with RNAseq (see ref). Clone #125 grows tight monolayers on plastic (tissue culture treated) and glass (very adherent). We observe strong DBA staining, demonstrates significant PC2 knockdown with induction (see western).
Antigen Expression	Antigen expression: This cell line is positive for epithelial cell marker Na+/K+/ ATPase (immunocytochemistry), and positive for distal nephron (principle cell marker) DBA.
Transwell Resistence	Forms very high resistence epithelial layer, TER= 2000 ohms*cm2 5 days after confluence.
Morphology on plastic	Forms tight epithelial layer and occassional small "domes" implies vectorial transcellular transport
Growth in 3D culture	Forms speroids exclusively (see reference Dixon et al. below)

Polycystin expression

PC2 expression is significantly reduced six days after Dox application (see western above).

Culture Method

Complete Growth Medium

RenaLife / Advanced MEM Culture Media Recipe:
We use a 1:1 mix of RenaLife Complete Medium (Basal Medium and Additives pack or Complete Kit; Lifeline Cell Technology, # LL-0025) and Advanced MEM medium (Fisher Scientific, from Life Technologies, Invitrogen and Gibco-BRL, # 12492).

1. To the 500 ml bottle of RenaLife add the additives that come with the kit, except the included FBS.
2. Add 12 ml of Pen/Strep standard stock solution, 300 ul of gentamicin standard stock solution and 6 ml of standard stock solution of L-alanyl-Glutamine.
3. Combine with the 500 ml bottle of Advanced MEM in a 1 liter filter apparatus.
4. We add 50 ml of FBS to reach 5%. The 2.5 ml of FBS included with the kit may be included in the 50 ml. The CCEC does not recommend a higher % FBS than 5% in mixed cultures because fibroblast overgrowth may occur.
5. **For Immortalized cells only:** *Propagate at 33 C. At each feeding add 1ul of interferon- gamma standard stock solution per 10ml medium. Omit this for experiments at 37 C.*

Notes:

- Pen/Strep stock solution (Fisher Scientific, Cellgro Mediatech, Catalog #30-002-CI)
- L-alanyl-Glutamine stock solution (Gemini Bio-Products, Catalog #400-106-100ml), sold as Stabilized Glutamine
- Gentamicin stock solution (Fisher Scientific, Cellgro Mediatech, Catalog #30-005-CR)
- **For Immortalized cells only:** *Interferon-gamma for propagation only (Cell Signaling Catalog #39127, 100ug/ml). Use at 10ng/ml at 33C. Remove when performing experiments at 37C.*

- We also filter all of our media with filter bottle systems and optionally add low antibiotics to our Ca Mg-free PBS and to our trypsin-EDTA solution.
- Serum % can be increased to 10% if attachment is a critical factor.
- With these advances, primary cultures passage 3-8 times and immortal cell lines perform well.
- This medium is formulated for use with a 5% CO2 in air atmosphere.

Subculturing Protocol

Cultured Cell Protocol:
1. For propagation, grow the cells at 33ºC in media containing
2. On arrival of frozen cells, thaw quickly and dilute contents by
3. Allow cell's 2 days to attach
4. Change culture medium every 3-4 days, according to the density of the cells.
5. It's a good idea to propagate/ passage as many flasks as you reasonably can before moving on to experiments to insure a large supply of low passage cells.

For splitting cultures:
1. Aspirate off the growth medium.
2. Rinse well with Mg- and Ca-free PBS. Add 1.5 ml standard trypsin-EDTA solution to T75 or 0.75 ml to T25 flasks.
3. Incubate in the 33ºC incubator for 5 minutes, until the cells round up and release from the plastic.
4. Add 11 ml culture medium to T75 or 5 ml to T25 flasks.

5. [optional] Spin the cell suspension in a conical centrifuge tube at about 200xg for 3 minutes, then aspirate off the supernatant fluid.

6. Resuspend the cells in 10 ml of culture medium if propagating to the same size flask. Otherwise, adjust the concentration appropriately.

7. Add one ml of cell suspension to each new flask, followed by 11 ml of culture medium for T75 flasks or add 5 ml medium for T25 flasks. Initially, change the medium after 1-2 days following splitting.

For experimentation, use medium without interferon-gamma, and place the cells in a 37ºC incubator for 10 days to permit the cells to revert to their non-immortalized state. You can monitor the reversion by Western Blotting and look for loss of the large T-antigen, if desired. For phenotype development for experimentation, it is recommended that the cells be grown on collagen-coated filters (e.g. Corning COSTAR 3493)

Doxycycline treatment for Polycystin KO

1. Grow cells at 33C plus IFN-gamma. Move cells to 37C without IFN-gamma.

2. Make doxycycline stock: Prepare 12mg in 1ml DMSO (12ug/ul). We have used several forms of doxycycline with equal success.

3. After 10 days at 37C, add doxycycline (final 10ug/ml) at the rate of 0.833ul stock per ml of culture medium and an equal amount of DMSO, if necessary, to the control medium. It may be necessary to filter-sterilize the medium if the doxycycline and/or DMSO is not sterile. Change the medium every day. Allow 6 days for the floxed polycystin allele to substantially disappear from the cells.

4. Alternatively we have also had success with starting the doxycycline treatment during the 10 day conversion period, DOX added on the fourth day at 37C and continued for the next 6 days provides significant KO, 6 days earlier.

Cryopreservation

For Freezing:

For freezing, we follow the protocol above for T75 flasks, but instead of resuspending in culture medium, we resuspend the cells in 4 ml of C80EZ (Mammalian Cell Cryopreservation Media Cat. 501001) including 5% DMSO v/v. Add 1 ml of cell suspension to each of 4 cryovials and freeze slowly (about -1ºC/min). Store at -80C or long term at -130C.

Specifications

Volume	1.0 mL
DNA Finger printing	*NA*
Population Doubling Level (PDL) and karyotype information	ND

Reference

H.J. Jung, R.A. Coleman, E.E. Dixon, P.O. Outeda, O.M. Woodward, and P.A. Welling (2019) Identification of cystogeneic signaling pathways in a newly developed, inducible-kidney epithelial cell model of Pkd2-mediated PKD. American Society of Nephrology Abstracts

Eryn E. Dixon, Demetri S. Maxim, Victoria L. Halperin Kuhns, Allison C. Lane-Harris, Patricia Outeda, Andrew J. Ewald, Terry J. Watnick, Paul A. Welling, and Owen M. Woodward (2020) GDNF drives rapid tubule morphogenesis in a novel 3D in vitro model for ADPKD. Journal of Cell Science. Jun 8:jcs.249557.doi: 10.1242/jcs.249557. https://pubmed.ncbi.nlm.nih.gov/32513820/