1) Detection of mouse polycystin-1 by immunoprecipitation using anti-mCC antibodies.

Sample preparation

1) Lyse mouse tissues or cell pellet in lysis buffer (25 mM Sodium Phosphate pH7.2, 150 mM NaCl, 10 % Glycerol, 1 mM EDTA, 1% Triton, Protease Inhibitor (Roche:11873580001))

Note:
Use 1 ml lysis buffer for 0.1g of tissue or cell pellet from one confluent P150 plate.

2) Homogenize tissue by Polytron (PT-MP 2100, Kinematica AG). For cells, up-and-down pipetting is usually enough.

3) Rotating the sample for 1 hour at 4^{o}C; Spin at 10,000 rpm in a table centrifuge for 10 min to pellet cell debris and nuclei. Collect clear supernatant and measure the protein concentration.

Immunoprecipitation

4) For typical IP reaction, use 1-2 µl of chicken IgY mCC to 2 mg protein in 1 ml.

5) Rotate the sample for 2 hours at 4^{o}C.

6) Add 30 ul goat anti-chicken IgY-Agarose
(Aves Labs:cat# P-1010, wash with lysis buffer before use).

7) Rotate overnight at 4^{o}C.

8) Spin sample at 2,000 rpm for 2 min; discard the supernatant; Wash the agarose beads with lysis buffer 3 times

9) Add 50ul 1xSDS-Sample Buffer. Heat at 95^{o}C for 3-5 min before loading. 15-20ul is sufficient for one lane.

Western blot

Use 4% (home made) or 3-8% gradient SDS gels (commercially available e.g. at Invitrogen).

Use rabbit polyclonal mCC serum at 1:1000-2,000 dilution as primary antibody for detection.

Note: the mCC antibodies are specific for mouse (and probably also for rat) polycystin-1. They are not suitable for detecting human polycystin-1 due to modest degree of aa identity of the antigen region, the cytoplasmic C-terminal tail.

2) Detection of epitope-tagged polycystin-1.

The cell pellet (10^6 cells) with expression of FLAG-tagged PKD1 expression is resuspended in 1 ml of lysis buffer (20 mM sodium phosphate, 150 mM sodium chloride, 10% glycerol, 1 mM EDTA, 0.5% Triton-X 100, pH 7.2 and complete TM protease inhibitor cocktail (Roche) and set on ice for 1 h. Centrifuge at 10,000*g* at 4 °C for 15 min. The cleared lysate is incubated with 20 µl of M2–Flag conjugated agarose beads (Sigma) with gentle rotation at 4 °C for 1 h (or overnight). Wash the beads (along with the immunoprecipitate) five times with 1 ml of lysis buffer. The bound proteins are then eluted with 50 µl laemmli buffer. Fifteen microliters of the eluted products and an equal volume of whole-cell lysates were subjected to polyacrylamide gel electrophoresis (4% or 3-8% acrylamide for polycystin-1), and then analyze using standard western blot protocols.

For detection of Myc- (or HA-tagged) polycystin-1, add 2 µg of anti-Myc or -HA antibody (Roche) to the cleared lysate. Incubate for 1 hr to overnight with gentle rotation at 4 °C. Add 50 µl of protein G sepharose to the samples, and allow them to incubate for a second hour at 4 °C. Wash the immunoprecipitate five times with 1 ml of lysis buffer, then elute it with 50 µl of laemmli buffer. Analyze the immunopurified protein as described above.