

Cyst Swelling Assay for ADPKD



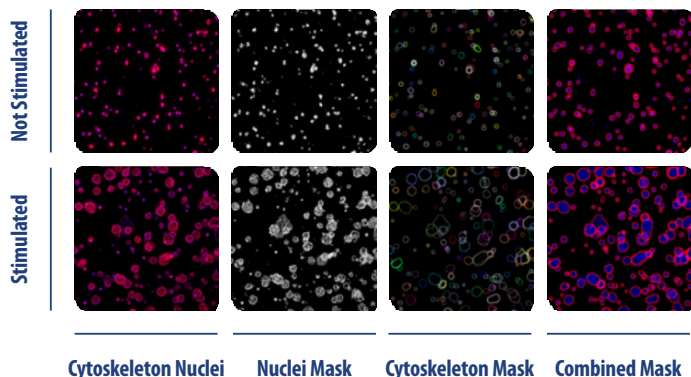
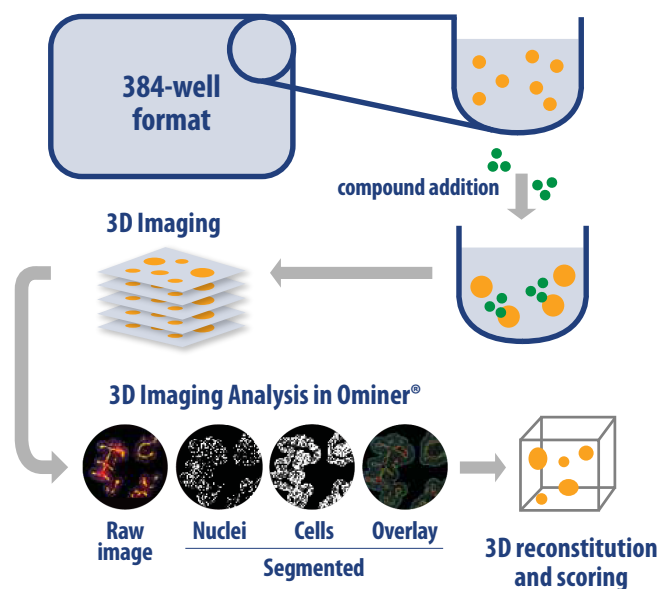
A unique high content screening platform for Autosomal Dominant Polycystic Kidney Disease (ADPKD) utilizing OcellO technology.

Polycystic kidney disease (PKD) is a genetic disorder that causes many fluid-filled cysts to grow in the kidneys. Our ADPKD patient-derived organoid cultures are made from materials collected from nephrectomy tissue from PKD patients. PKD cyst swelling can be measured using this unique assay that recapitulates physiologically relevant kidney cyst structures in a 3D *in vitro* screening platform.

This imaging-based assay using a 384-well plate 3D assay format enables the recapitulation of the *in vivo* disease phenotype with robust functional readouts.

Key advantages:

- High content phenotypic evaluation by 3D imaging
- Sensitive and robust functional readouts
- Separation of toxic from effective compounds
- Optimal *in vitro* to *in vivo* translation
- Scalable assays include a robust cell-line-derived cyst assay for high-throughput screening and an *ex vivo* assay using patient-derived cystic tissue



Following pre-culture, cells are seeded in an extracellular matrix in 384-well plates, where they spontaneously form cysts, typically within 1-4 days. Cyst swelling is stimulated by addition of compounds to induce swelling, such as vasopressin or forskolin. Simultaneously, treatment to inhibit swelling is added for 48-72 hours. Setup can be customized depending on research questions.

PKD Cyst Assay Factsheet

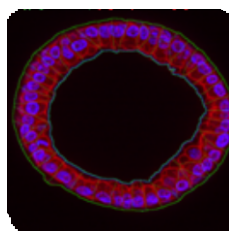


Functional Readouts

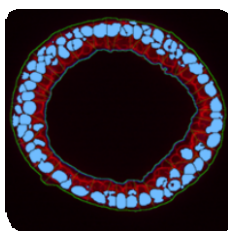
Murine Cell Line Model of ADPKD

- Murine Pkd1^{-/-} collecting duct cells develop into cysts in 3D culture
- cAMP-dependent cyst swelling is induced by forskolin or prostaglandin E2
- Test compounds are added and the effect on cyst swelling is determined
- This robust model is suited to high-throughput screening and enables detailed phenotypic analysis of treatment effect and the discrimination between efficacy and toxicity

Cyst in 3D culture



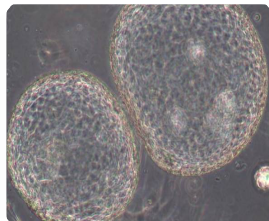
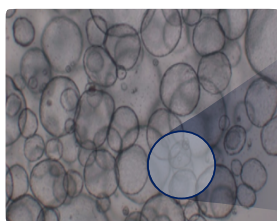
Nuclei / Actin



Segmented

ADPKD Patient-Derived Ex Vivo Cultures

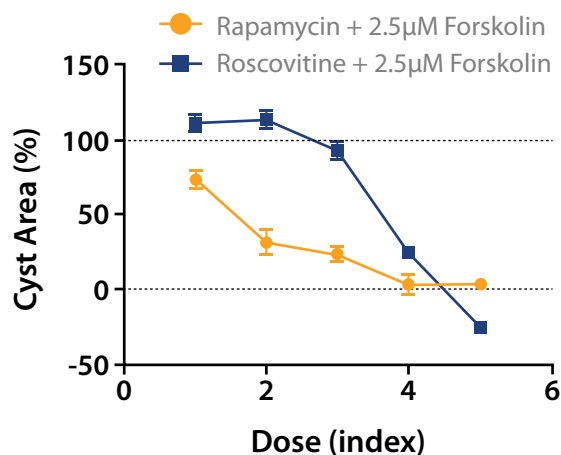
This ex vivo assay uses material collected from nephrectomy tissue from PKD patients. These tissues form cysts when cultured in a 3D environment. The cultures are biobanked at low passage numbers for subsequent compound testing. Cyst swelling can be driven by the addition of agonists such as desmopressin or forskolin.



Culture of ADPKD Patient Material

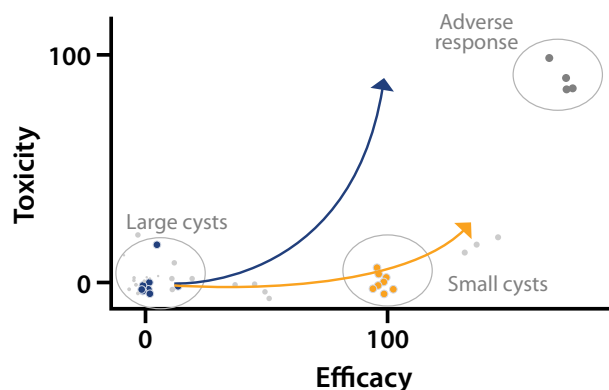
Robust Functional Readout

Pkd1^{-/-} mouse collecting duct cells were cultured in 3D. After four days, cultures were exposed to forskolin and reference compounds for 72 hours. Their capability to inhibit cyst swelling was compared to solvent control (0.2% DMSO) and stimulant only control (forskolin).



Discriminating Between Inhibition of Cyst Swelling and Toxicity

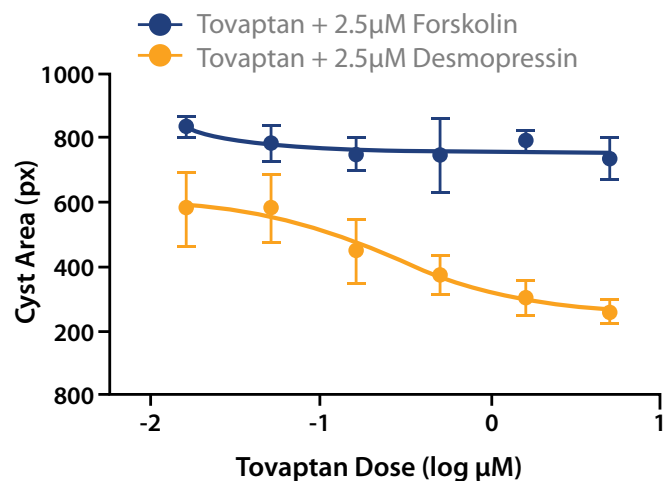
Analysis of multiple morphological features associated with inhibition of cyst swelling and cytotoxicity and nuclear toxicity enables the discrimination of compounds with high therapeutic potential (gold arrow) compared to those which are associated with adverse toxic responses (blue arrow).



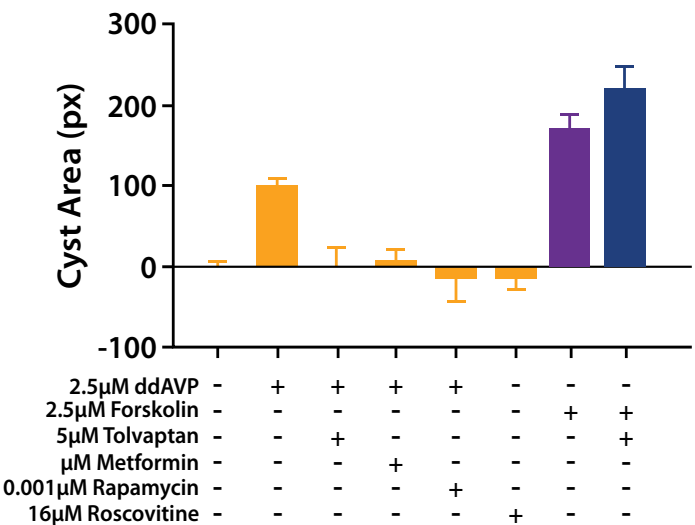
PKD Cyst Assay Factsheet



In Vivo-like Drug Responses



Cells harvested from ADPKD patients were seeded in 384-well plates in 3D *in vitro* culture. After 24 hours, cultures were exposed to stimulant with reference compounds for 48 hours. Forskolin (blue) or desmopressin (gold) in combination with tolvaptan (data not normalized) shown.



Reference compounds were compared to positive control (solvent only) and negative control (desmopressin (ddAVP) only or forskolin only) for their capability to inhibit cyst swelling. Data was normalized to solvent only (0%) and desmopressin (ddAVP) only (100%).

Contact Sales
US: +1 855 827 6968
UK: +44 870 166 6234
busdev@crownbio.com

Schedule Scientific Consultation
Request a consultation to discuss your project.
consultation@crownbio.com

Explore Scientific Data
Contact us to learn more about ADPKD models and cyst assays.
busdev@crownbio.com