Renal Proximal Tubule Epithelial Cells
A2 Clone - Expression of Uptake and Efflux Transporters

Genetically Engineered Primary Cells
Sigma Life Science has utilized its CompoZr® Zinc Finger Nuclease (ZFN) technology to engineer primary Renal Proximal Tubule Epithelial Cells (RPTECs) that provide a robust and predictive cell line for in vitro ADME/Tox studies. A key factor that makes this cell line ideal for cytotoxicity studies is the expression of major uptake and efflux transporters including, OAT1, OAT3, OCT2, MDR1, MRP2, MRP4 and MATE2.

The A2 Clone also demonstrates normal epithelial morphology in culture relative to primary RPTECs.

Uptake Transporter Functional Data

OAT1/3

Figure 1.
The uptake of P-Aminohippuric acid (PAH) was tested in the presence and absence of Probenicid. The uptake of PAH was significantly reduced in the presence of Probenicid, indicating OAT1/3 are functional in the A2 Clone.

OCT2

Figure 2.
The OCT2 uptake transporter was tested by the accumulation of Tetraethylammonium (TEA). A dose-response effect is observed which further validates the presence of functional OCT2 in the A2 Clone.

Efflux Transporter Functional Data

MDR1 (P-gp)

Figure 3.
The efflux of Calcein-AM was tested by measuring fluorescence with and without the addition of Cyclosporine-A which is an MDR1 inhibitor. At two different concentrations the RFUs were significantly higher in the presence of Cyclosporine-A.

MRP2

Figure 4.
Fluorescence of 5(6)-Carboxy-2'-7'-dichlorofluorescein (CDCF) was measured by accumulation in the presence and absence of MK571. Higher RFUs were observed in the presence of MK571 which demonstrates the inhibition of the MRP2 transporter.
Characterization of the A2 Clone
Several assays were conducted to confirm the presence of the appropriate brush-border membrane proteins including aminopeptidase N, α-GST and γ-Glutamyl transferase. Other assays to confirm the A2 Clone as RPTECs include albumin uptake and cAMP stimulation in the presence of parathyroid hormone. In addition to this functional data the A2 Clone has substantially equivalent morphological characteristics in culture relative to primary RPTECs.

Expression of PT-specific Markers

![Negative Control](CD13_neg.png) ![Positive Control](CD13_pos.png)

**Figure 5.** The presence of the brush-border membrane protein aminopeptidase N was detected in the A2 Clone by using the mouse anti-human CD13 fluorescent protein. Human kidneys contain high levels of glutathione S-transferases (GST) with α-GST being solely present in the proximal tubular cells. Fluorescent staining displays the presence of α-GST in the A2 Clone.

Albumin Uptake

![Figure 6.](Albumin_Uptake.png)

**Figure 6.** The reabsorption of albumin was analyzed in confluent monolayers using fluorescein isothiocyanate (FITC) labeled bovine serum albumin (BSA). The temperature dependence of BSA uptake is displayed as both the WT and A2 Clones have higher uptake at 37 °C relative to 4 °C.

γ-Glutamyl Transferase Activity

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**Figure 7.** The enzyme γ-Glutamyl transferase (GGT) is located in the brush border of the tubule and is considered to be a characteristic marker for RPTECs. GGT catalyzes the removal of the γ-GT moiety from γ-GT/pNA leading to the release of P-nitroanilide (pNA). These data demonstrate substantial equivalence between the WT and A2 Clone for the release of pNA as determined by spectrophotometry.

Response to Parathyroid Hormone

![Figure 8.](Response_to_PTH.png)

**Figure 8.** In vivo RPTECs respond to parathyroid hormone (PTH) by stimulating cAMP production. These data demonstrate the stimulation of cAMP production in the A2 Clone at various concentrations of PTH measured at 15 minute and 30 minute time points.

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