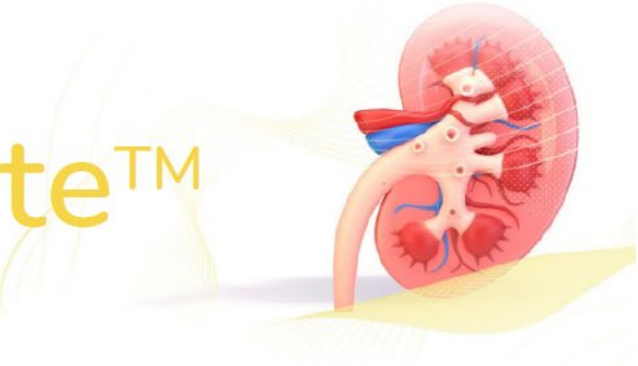
# aProximate™



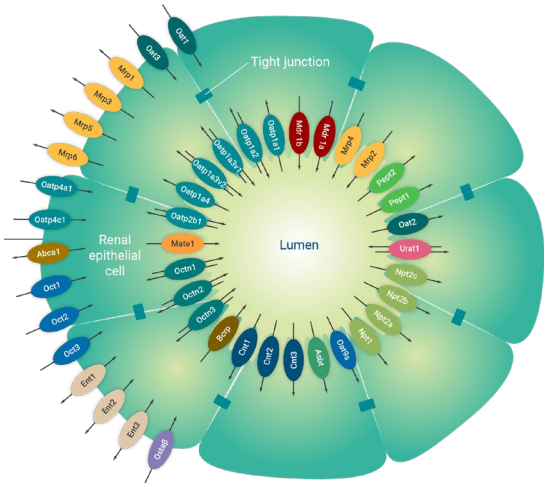
KIDNEY

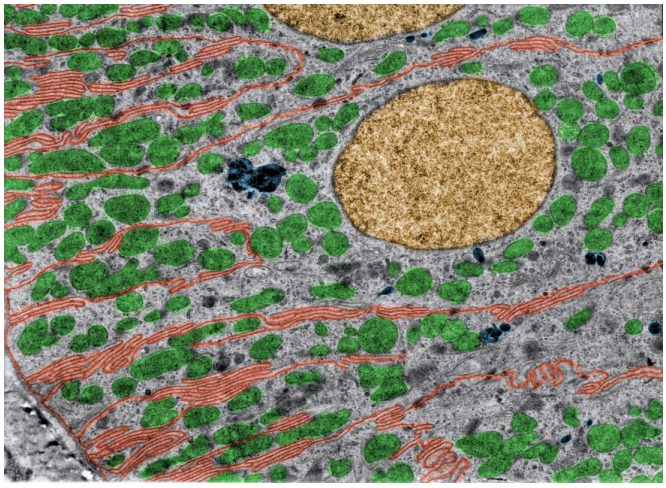
# Mitochondrial health assays in Kidney Proximal Tubules (PTCs)

## Overview

Kidney Tubule Cells (PTCs) contain a large number of mitochondria to handle drugs and excrete xenobiotics. Mitochondrial health is a good indicator of overall cell heath and allows the evaluation of drug safety.

- The kidney removes waste and toxins from the body.
- Proximal Tubule Cells (PTCs) are responsible for the reabsorption of solutes and the excretion of xenobiotics.
- To do so PTCs express a large number of transporters and contain a large number of mitochondria.



*Coloured Transmission electron microscopy (TEM) of a kidney tubule cells. Basal infoldings are labelled in red colour, mitochondria in green, and lysosomes in blue*

- Mitochondria generate the energy (ATP) that keeps the cells healthy and able to function.
- Some drugs affect mitochondrial function causing damage to PTCs and even the loss of renal function.
- For new drugs, it is recommended to monitor mitochondrial health in PTCs and to avoid drugs inducing cell damage.

Drugs can affect cell metabolism and mitochondrial function. New drugs are required to be tested *in vitro* to assess how they are handled by PTCs and whether they may cause damage to the cell and affect mitochondria. Reliable models are needed to test the effect of new drugs on mitochondrial function.

Newcells provides an assay service to monitor mitochondrial health in kidney PTCs.

## Three assays to check mitochondrial health

- Mitochondrial Function – **Agilent Seahorse™ XF Assay**
- Mitochondrial Membrane Potential (MMP) – tetramethyl rhodamine methyl ester assay
- Oxidative Stress – Measurement of reactive oxygen species (ROS) production

Best in Class *in vitro* models to most accurately predict *in vivo* outcomes


newcellsbiotech.co.uk

The Biosphere, Newcastle Helix, Newcastle Upon Tyne NE4 5BX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## Mitochondrial function using the Agilent Seahorse™ XF assay

Normal cells rely on mitochondrial oxidative phosphorylation (OXPHOS) to produce ATP as a source of energy. The Seahorse™ XF assay measures the oxygen consumption rate (OCR) and pH or extracellular acidification rate (ECAR) in the media surrounding living cells. It is a fast, real-time, label-free, highly sensitive and extremely precise measurement of cellular metabolism that correlates with mitochondrial function.

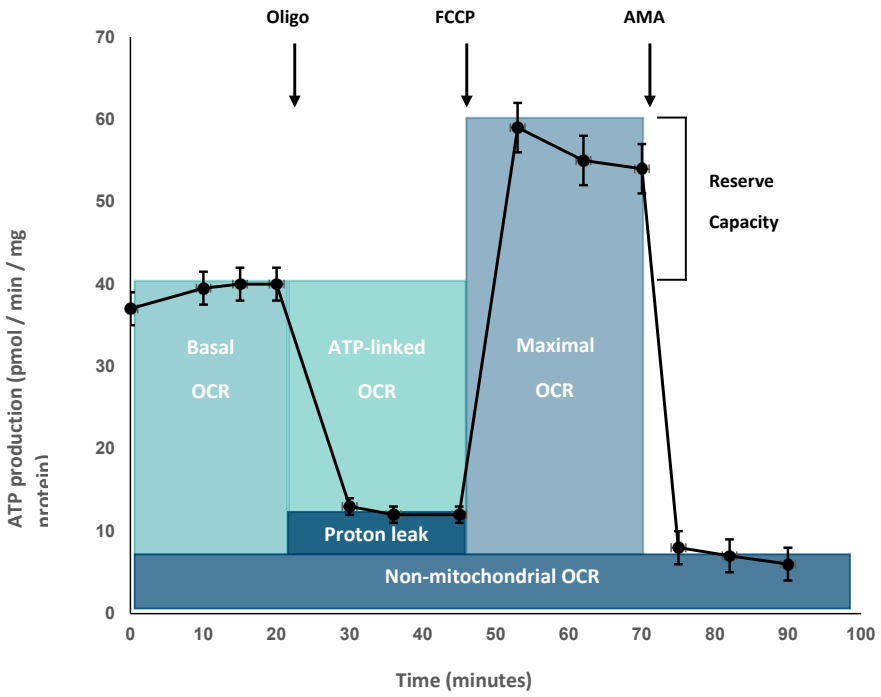
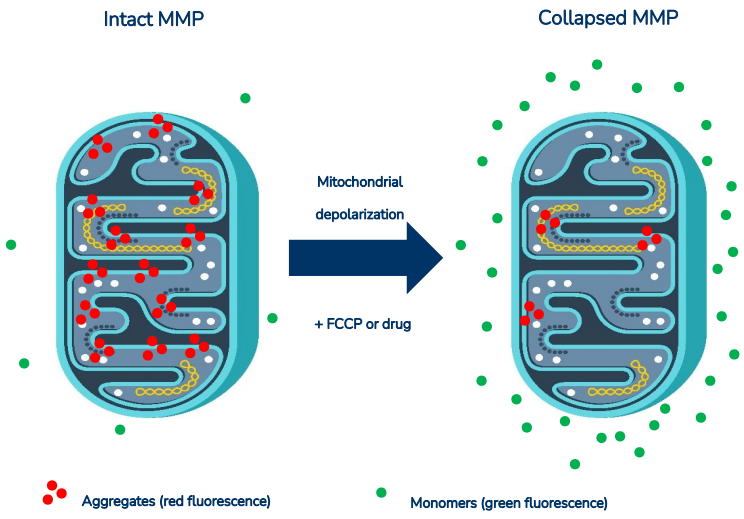



**FIGURE: Mitochondrial bioenergetics explained.** Basal OCR drops when ATP production is stopped by the addition of ATPase inhibitor Oligomycin, leaving proton leak and non–mitochondrial OCR. Addition of the uncoupler FCCP restores maximal respiration, whilst addition of inhibitor Antimycin A (AMA) blocks mitochondrial oxygen consumption.

## Mitochondrial Membrane Potential (MMP) assay




Mitochondrial function can also be assessed by monitoring changes in mitochondrial membrane potential (MMP). Preservation of mitochondrial membrane potential is a key indicator of cell health. MMP assay is based on the measurement of tetramethyl rhodamine methyl ester accumulated in the mitochondria and it is an indicator of cell health status. Mitochondrial depolarization occurs as a result of an uncoupling compound (FCCP) or drug affecting the mitochondrial membrane potential (MMP).

**FIGURE: MMP assay principle.** In healthy cells the mitochondrial membrane potential indicator (MPI) dye accumulates in mitochondria as aggregates (red fluorescence can be detected). When mitochondrial potential collapses MPI dye is mainly distributed in the cytoplasm as monomers (green fluorescence).

## Oxidative Stress assay

Oxidative stress is a phenomenon caused by the imbalance between production and accumulation of reactive oxygen species (ROS) in the cells. ROS are produced as a result of mitochondrial damage by some drugs and xenobiotics. ROS production, measured using a fluorescent probe, gives an indication of cell health status.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