Endocrine Disruption

**In vitro Androgen Receptor Modulation Assay**

**Background Information**

- The androgen receptor (AR) is a nuclear receptor activated by binding either testosterone or dihydrotestosterone in the cytoplasm and translocating to the nucleus.
- Environmental contaminants such as industrial and agricultural chemicals have been shown to alter androgen receptor (AR) function through agonistic or antagonistic modulation.
- Androgen disruptors can lead to reduced sperm count, infertility, prostate cancer and can interfere with normal male development.
- AR agonists and some antagonists induce nuclear translocation. This can be detected using GFP-tagged proteins which are monitored in the cytoplasm and nucleus of the cells. The assay can also detect nuclear foci (punctuate distribution in the nucleus) which is characteristic of AR agonists.
- The in vitro Androgen Receptor (AR) modulation assay can be applied as a therapeutic screening assay (e.g., in the development of prostate cancer therapies) or to assess potential endocrine disruption effects.

**Protocol**

**Test System**
AR Redistribution Assay (ThermoScientific)

**Cell Type**
Recombinant U2OS cells stably expressing human androgen receptor

**Test Article Concentration**
10 µM in quadruplicate (alternative concentrations or dose curves available on request)

**Test Article Exposure**
Overnight

**Reference Controls**
Dihydrotestosterone (agonist)
Mifepristone (antagonist)

**Typical Vehicle Control**
0.5% DMSO

**Analysis Method**
ThermoScientific ArrayScan® VTi High Content Imaging

**Data Delivery**
% Effect compared to control (single concentration)
EC_{50} (dose response)

*The AR is a ligand-dependent transcription factor that controls the expression of specific genes. The binding of the AR to its native ligands 5α-dihydrotestosterone (DHT) and testosterone initiates male sexual development and differentiation.*

Both the agonist dihydrotestosterone and the antagonist mifepristone induce nuclear translocation of AR. However, dihydrotestosterone can be identified as an agonist by the punctuated distribution pattern in the nuclei (also known as nuclear foci).

The data in Figure 2 show the reproducibility of the positive control agonist (dihydrotestosterone) and negative vehicle control across eight different plates (different colours represent different plates) and across the same plate (same colours represent sixteen positive control wells and sixteen negative control wells within the same plate).

Mean_CircRingAvgIntenDiff is the difference between the average intensity of the nucleus (circle) and the cytoplasm (ring) of the cell.

The data in Figure 3 show the reproducibility of the positive control antagonist 17-AAG (17-allylamino-17-demethoxygeldanamycin) and negative vehicle control across eight different plates (different colours represent different plates) and across the same plate (same colours represent sixteen positive control wells and sixteen negative control wells within the same plate).

Mean_CircRingAvgIntenDiff is the difference between the average intensity of the nucleus (circle) and the cytoplasm (ring) of the cell.

References