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Endocrine Disruption

In vitro Androgen Receptor Modulation Assay

Background Information

'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.'

[2]Tan MHE *et al*., (2015) Androgen receptor: structure, role in prostate cancer and drug discovery. *Acta Pharmacologica Sinica* **36**; 3-23

- The androgen receptor (AR) is a nuclear receptor activated by binding either testosterone or dihydrotestosterone in the cytoplasm and translocating to the nucleus[1].
- 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

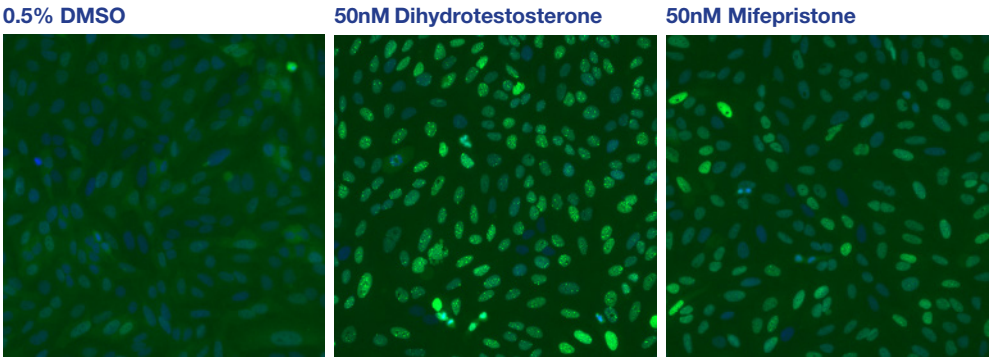
Analysis Method
ThermoScientific ArrayScan® VTi High Content Imaging

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

'The AR redistribution assay is a robust screening assay for the detection of androgen receptor modulators that can be run in either agonist or antagonist mode.'

Figure 1

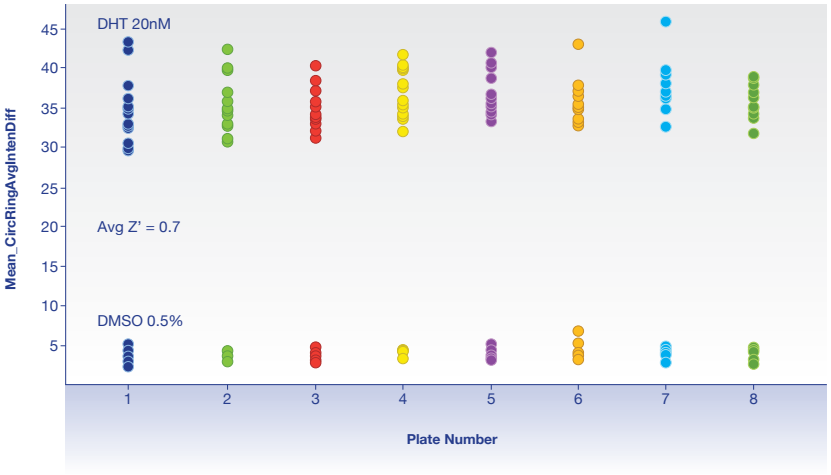
High content screening images illustrating the effect of the agonist dihydrotestosterone and the antagonist mifepristone on the translocation of AR-GFP into the nucleus



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).

Figure 2

Spotfire visualisation of reproducibility of the vehicle control (0.5% DMSO) and dihydrotestosterone (20nM) in agonist mode.

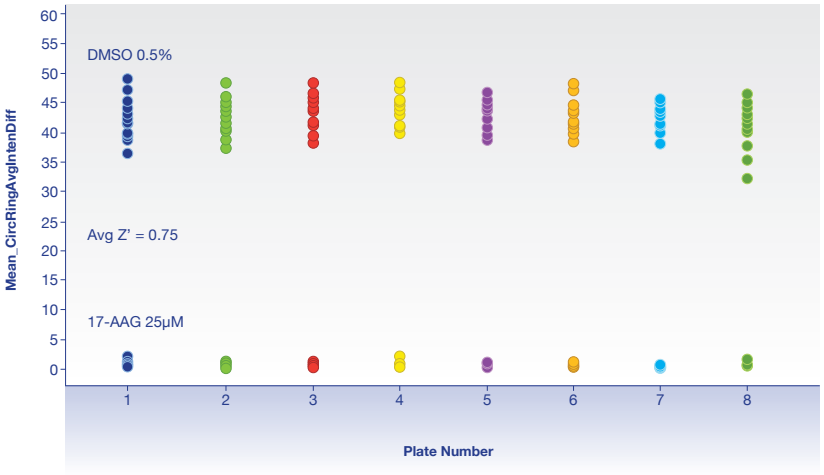


The data in Figure 2 show the reproducibility of the positive control agonist (dihydrotestosterone) and negative vehicle control across eight different plates (different colours represent different plates) and across the same plate (same colours represent sixteen positive control wells and sixteen negative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

Figure 3

Spotfire visualisation of the vehicle control (0.5% DMSO) and 17-AAG (25μM) in antagonist mode (in the presence of dihydrotestosterone).



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

[1] Hua Y, *et al*, (2014) High-content positional biosensor screening assay for compounds to prevent or disrupt androgen receptor and transcriptional intermediary factor 2 protein-protein interactions. *ASSAY Drug Dev Technol* **12(7)**; 395-418

[2] Tan MHE *et al*., (2015) Androgen receptor: structure, role in prostate cancer and drug discovery. *Acta Pharmacologica Sinica* **36**; 3-23