In Vitro Steroidogenesis Assay

Need

Endocrine disruption is of regulatory importance globally and is a primary consideration in determining the safety and/or efficacy of a dermatological drugs, cosmetics, chemicals, and agrochemicals. Multiple enzymes catalyze a series of reactions, resulting in the conversion of cholesterol to the hormones testosterone and estradiol, in a process called steroidogenesis. Compound or chemical perturbation of normal steroidogenesis may have adverse effects on normal development, reproductive health, and/or the integrity of the reproductive system. For new and existing drugs, cosmetics, chemicals, and agrochemicals, the cost and time necessary to assess disruption of steroidogenesis *in vivo* is prohibitory to efficient product development and safety assessment.

Solution

The potential for a compound or chemical to affect steroidogenesis can be assessed early in the product development cycle to determine their relative risks for causing endocrine disruption. LifeNet Health LifeSciences offers the validated Steroidogenesis assay under OECD Test Guideline 456 for the assessment of potential induction, or inhibition, of steroidogenesis by test compounds or chemicals. In our Steroidogenesis, the H295R cell line is used. This cell line expresses genes that encode all the key enzymes needed for the production of testosterone and estrogens (Figure 1) and therefore is a unique model system to assess disruption in the production of these hormones. This assay is performed for both agonism (induction of testosterone and/or estrogen production) and antagonism (inhibition of testosterone and/or estrogen production). The presence of testosterone and 17β-estradiol in the cell culture media is determined by LC-MS/MS analysis using validated methods. Based on these results, it can be predicted whether the test material has an effect (induction or inhibition) on the steroidogenic pathway. At least two runs are conducted for each study.

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Standard Protocol

ASSAY PARAMETERS	PROTOCOL
Model	H295R cell line
Replicates	3
Solvent of Choice	DMSO (preferred) or sterile water
Test Article Formulation	300, 100, 30, 10, 3, 1 and 0.3 µM (depending on solubility and cytotoxicity pre-testing)
Solvent Controls	DMSO (or sterile water)
Agonism Positive Control	10µM Forskolin
Antagonism Positive Control	1 µM Prochloraz
Exposure Time	24 ± 2 hours
Cell Viability Assessment	MTT assay
Estrogen and Testosterone Concentration	LC-MS/MS
Time to Complete	4-6 weeks from test article receipt
Regulatory	Non-GLP or GLP
Deliverables	Full Report including: categorization (positive, negative, or equivocal for hormone induction). The EC20, EC50, and lowest observed effective concentration (LOEC) will be provided, if possible.

Key References

OECD (2023), Test No. 456: H295R Steroidogenesis Assay, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264122642-en.

Figure 1. The steroidogenesis pathway

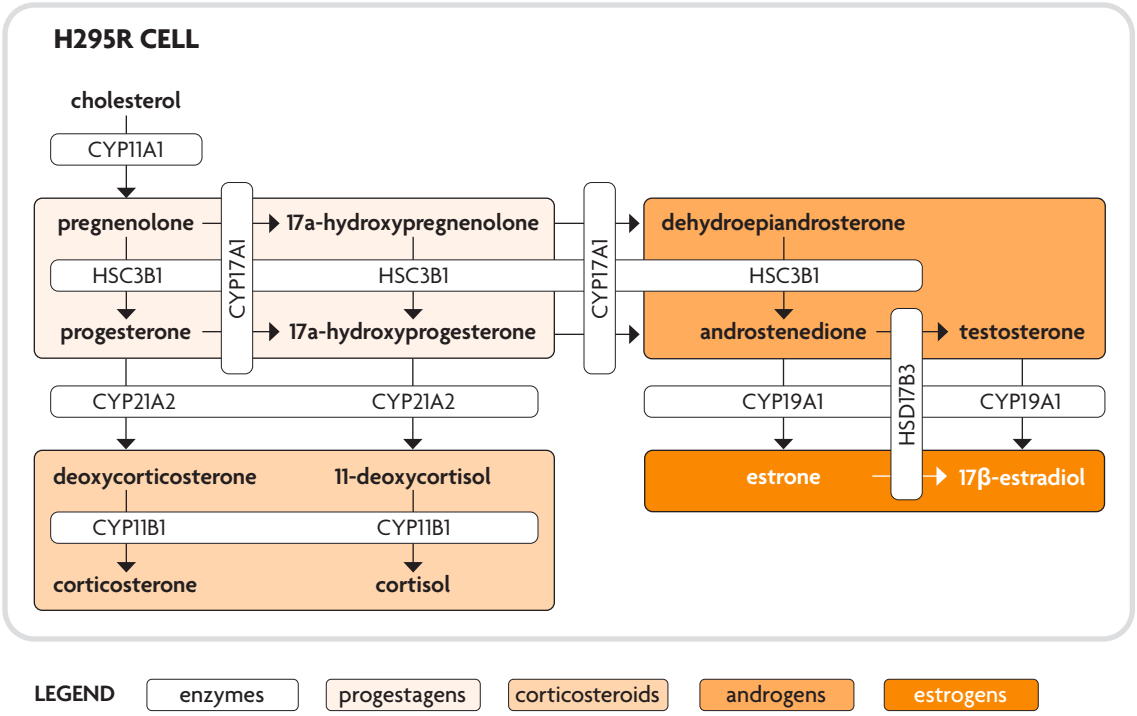

Figure 2. Positive and Negative decision criteria