

## H295R assay

The in vitro H295R Steroidogenesis Assay (H295R) described in OECD test guideline 456 (OECD, 2011) utilises human adenocarcinoma cell line NCI-H295R. In this cell line, the human steroidogenesis pathway is fully functional. Upon exposure of this cell line to a test compound, the effect on steroidogenesis can be assessed by quantifying the amount of 17 $\beta$ -estradiol (E2) and testosterone (T) produced by the cells and comparing this to cells exposed to vehicle control only. At BDS, the quantification of E2 and T is performed using the AR CALUX and ER $\alpha$  CALUX bioassays. The goal of the assay according to the OECD guideline is to provide a YES/NO answer with regard to the potential of a chemical to induce or inhibit the production of T and E2; however, it is also possible to report quantitative results by determining a lowest observed effect concentration (LOEC).

Specification	H295R assay
Basal cell line	H295R
Species	human
Tissue	adrenal
Positive control	17 $\beta$ -estradiol and testosterone
Endpoint (pure compounds)	EC or PC concentration, lowest effect concentration (e.g. PC10)
Endpoint (mixtures)	Toxic equivalents in pg TEQ/g sample processed
Test duration	48hr (incubation time)
Specificity	Interaction with endogenous steroidogenesis pathways
Matrices	Any type of sample
Sample volume/mass	Matrix- and desired limit of quantification (LOQ)-dependent
Amount of compound	Typically 10 mg. Lower for high potency compound provided in DMSO
Assessment criteria	In house methods, compliant with relevant application/regulations.
SOPs and Guidelines	BDS internal
HTS protocol	Not available yet
Key reference	Ouedraogo G, Alexander-White C, Bury D, Clewell HJ 3rd, Cronin M, Cull T, Dent M, Desprez B, Detroyer A, Ellison C, Giammanco S, Hack E, Hewitt NJ, Kenna G, Klaric M, Kreiling R, Lester C, Mahony C, Mombelli E, Naciff J, O'Brien J, Schepky A, Tozer S, van der Burg B, van Vugt B, Stuard S, Cosmetics Europe (2022) Read-across and new approach methodologies applied in a 10-step framework for cosmetics safety assessment - A case study with parabens. Regul Toxicol Pharmacol. 2022 May 1:105161.