

Mineralocorticoid Receptor Transactivation Assay (MR TA)

GR and MR mediate the actions of glucocorticoids and mineralocorticoids, respectively, which are two main classes of corticosteroids involved in many physiological processes. EDCs interfere with GR/MR activity by disrupting ligand/DNA-receptor binding, GR/MR expression and translocation (Zhang and al., 2019).

References

- Neale P, Grimaldi M, Boulahtouf A, Leusch F*, Balaguer P*. Assessing species-specific differences for nuclear receptor activation for environmental water extracts. *Water Research* 2020; 185 : 116247.
- Dellal H, Boulahtouf A, Alaterre E, Cuenant A, Grimaldi M, Bourguet W, Gongora C, Balaguer P*, Pourquier P.* High content screening using new U2OS reporter cell models identifies harmol hydrochloride as a selective and competitive antagonist of the androgen receptor. *Cells* 2020; 9: 1469.
- Grimaldi M, Boulahtouf A, Toporova L, Balaguer P. Functional profiling of bisphenols for nuclear receptors. *Toxicology*. 2019, 420: 39-45.
- Leusch FDL, Neale PA, Arnal C, Aneck-Hahn NH, Balaguer P, Bruchet A, Esther BI, Esperanza M, Grimaldi M, Leroy G, Scheurer M, Schlichting R, Schriks M, Hebert A. Analysis of endocrine activity in drinking water, surface water and treated wastewater from six countries. *Water Res* 2018, 139: 10-18.
- Bellet V, Hernandez-Raquet G, Dagnino S, Seree L, Pardon P, Bancon-Montiny C, Fenet H, Creusot N, Aït-Aïssa S, Cavailles V, Budzinski H, Antignac JP, Balaguer P. Occurrence of androgens in sewage treatment plants influents is associated with antagonist activities on other steroid receptors. *Water Res*. 2012 ; 46 : 1912-22.
- Escande A, Servant N, Rabenoelina F, Auzou G, Kloosterboer H, Cavailles V, Balaguer P, Maudelonde T. Regulation of activities of steroid hormone receptors by tibolone and its primary metabolites. *J Steroid Biochem Mol Biol*. 2009 Aug;116(1-2):8-14.
- Molina-Molina JM, Hillenweck A, Jouanin I, Zalko D, Cravedi JP, Fernandez MF, Pillon A, Nicolas JC, Olea N, Balaguer P. Steroid receptor profiling of vinclozolin and its primary metabolites. *Toxicol Appl Pharmacol*. 2006; 216: 44-54.

Principle of the method

To characterize the human mineralocorticoid receptor (hMR) activity of chemical, a stable cell line expressing the ligand binding domain (LBD) of human MR fused to the yeast GAL4 DNA binding domain (DBD) was developed. This reporter cell line was generated by a two-step transfection procedure. U2OS cells are stably transfected by a GAL4 (DBD)-hMR (LBD) plasmid and then by a luciferase plasmid under the control of GAL4 promoter (GAL4-Luciferase). This stable model allows specific and sensitive measurement of hMR ligand activities thanks to the use of luciferase, and is a high-throughput, cell-based screening tool for identifying and characterizing hMR ligands. This test elucidates the Molecular Initiating Event of the activation/inhibition of the MR receptor and should be included in an Integrated Testing Strategy (ITS)/IATA for the regulatory identification of a substance as an endocrine disrupter (*figure*). The cells are available upon signature of an MTA.

Readouts : human mineralocorticoid receptor (hMR), reporter gene

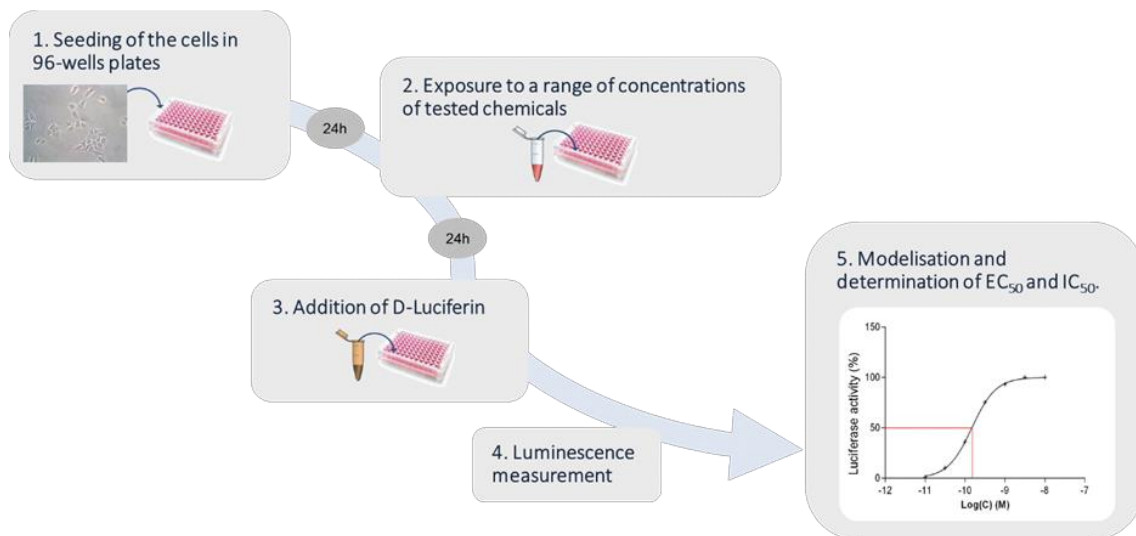


Figure : Throughput of the assay. Reading of the luminescence is optimal 20 minutes after the addition of the luminescence medium.

Necessary equipment and consumables

- **Standard cell culture equipment** (e.g. incubator, laminar flow hood)
- **Plate reader which can measure luminescence, no need of injectors** (e.g. MicroBeta Wallac luminometer (Perkin-Elmer))
- **96 wells white opaque culture plates** (e.g. Greiner bio-one 655083-905, CellStar; Dutscher, Brumath, France)
- **Cell cytotoxicity measurement** (e.g. Alamar blue, or Neutral red)
- DMEM/F-12 without phenol red (e.g. Gibco 21041-025) Test medium - 5% DCC-treated FBS
- 1% v/v penicillin/streptomycin (e.g. Gibco 15070-63)

Culture medium	<ul style="list-style-type: none"> - DMEM/F-12 with phenol red (e.g. Gibco 31331-028) - 10% FBS (e.g. Eurobio CVFSVF00) - 1% v/v penicillin/streptomycin (e.g. Gibco 15070-63) - 0.5 µg/ml puromycin (e.g. Sigma-Aldrich) - 1 mg/mL geneticin (e.g. Invivogen ant-gn)
Test medium	<ul style="list-style-type: none"> - DMEM/F-12 without phenol red (e.g. Gibco 21041-025) - 5% DCC-treated FBS - 1% v/v penicillin/streptomycin (e.g. Gibco 15070-63)
Luminescence medium	<ul style="list-style-type: none"> - DMEM/F-12 without phenol red (e.g. Gibco 21041-025) - 5% DCC-treated FBS - 1% v/v penicillin/streptomycin (e.g. Gibco 15070-63) - 0.3 mM D-luciferin (e.g. Perkin Elmer 122799)