Progesterone Receptor Transactivation Assay (PR-TA)

References

Grimaldi M, Boulahtouf A, Toporova L, Balaguer P. “Functional profiling of bisphenols for nuclear receptors”. Toxicology (2019) 420:39. DOI: 10.1016/j.tox.2019.04.003

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) 85:116247. DOI: 10.1016/j.watres.2020.116247

Garoche C, Aït-Aïssa S, Boulahtouf A, Creusot N, Hinfray N, Bourguet W, Balaguer P, Brion F. “Human and Zebrafish Nuclear Progesterone Receptors Are Differently Activated by Manifold Progestins”. Environ Sci Technol (2020) 54:9510. DOI: 10.1021/acs.est.0c02056

Kocour Kroupová H, Grimaldi M, Šauer P, Bořík A, Zálohová K, Balaguer P. “Environmental water extracts differentially activate zebrafish and human nuclear progesterone receptors”. Sci Total Environ (2023) 859:160232. DOI: 10.1016/j.scitotenv.2022.160232

Summary of the method

Progesterone is important for various processes and bodily functions including pregnancy, neuroprotection, behavioral development, bone formation, and metabolism. Often this is in collaboration with other hormones. Moreover, disruption of progesterone receptor (PR) signaling is associated with adverse outcomes such as an increased risk of developing hormone-dependent cancers.

This in vitro method measures the potential of chemical substances to activate or inhibit the PR through a luciferase reporter gene. Similar to other transactivation assays, PRTA relies on the binding and activation of chemicals to PR to induce the luciferase. In addition, the method can be turned into an antagonistic assay using progesterone that may be competed by test chemicals.

Readout and Result

The assay is based on the human osteosarcoma cells (U2OS) stably expressing the human progesterone receptor (hPR) together with a luciferase reporter construct. This stable model allows transactivation assessment of activation of hPR measuring luciferase activation, enabling identification of PR agonists and antagonists.

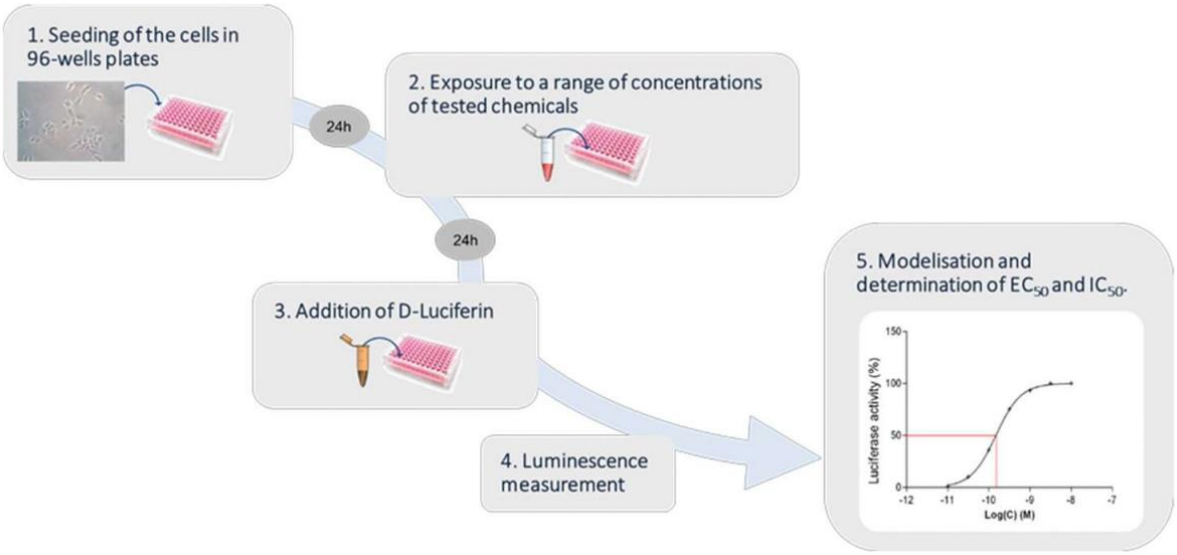

Figure 1: Workflow of the PRTA*. After seeding of the cells in 96-well plates, they are treated with a test compound for 24 hours. Thereafter, D-Luciferin is added, and Luminescence is measured.*

Necessary equipment

- standard cell culture equipment (e.g. incubator, laminar flow hood)
- 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 (Gibco 21041-025) Test medium
  - 5% DCC-treated FBS
  - 1% v/v penicillin/streptomycin (Gibco 15070-63)

Culture medium	- DMEM/F-12 with phenol red (Gibco 31331-028) - 10% FBS (Eurobio CVFSVF00) - 1% v/v penicillin/streptomycin (Gibco 15070-63) - 1 mg/mL geneticin (Invivogen ant-gn)
Test medium	- DMEM/F-12 without phenol red (Gibco 21041-025) - 5% DCC-treated FBS - 1% v/v penicillin/streptomycin (Gibco 15070-63)
Luminescence medium	- DMEM/F-12 without phenol red (Gibco 21041-025) - 5% DCC-treated FBS - 1% v/v penicillin/streptomycin (Gibco 15070-63) - 0.3 mM D-luciferin (Perkin Elmer 122799)

- Luminometer
  - Example : MicroBeta Wallac luminometer (Perkin-Elmer). Reading of microplates, from above.
  - Reading is optimal 20 minutes after the addition of the luminescence medium.