Human Peroxisome Proliferator-Activated Receptor γ Transactivation Assay (hPPARγ-TA)

References

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Summary of the method

The peroxisome proliferator-activated receptor γ (PPARγ) is a well-known regulator of adipocyte differentiation, immune responses, proliferation of different tissues, and metabolism including fat storage. PPARγ has been the target of drug discovery projects for a long period. These medicines include glitazones which enhance insulin sensitivity. There are numerous other metabolites and xenobiotics able to interfere with binding to PPARs. However, due to the obligatory heterodimerization of PPAR with the retinoid X receptor (RXR), it is complicated to distinguish effects caused by PPAR ligands from effects caused by RXR ligands. Moreover, dysregulation of PPARγ signaling is associated with the development of several tumors.

This in vitro method measures the specific activation or inhibition of chemical substances on the human PPARγ through a luciferase reporter gene. Similar to other transactivation assays, PPARTA relies on the binding and activation of chemicals to PPARγ to induce the luciferase. This can be turned into an antagonistic assay using rosiglitazone that may be competed by test chemicals.

Readout and Result

This test elucidates the Molecular Initiating Event of the activation/inhibition of hPPARγ. The assay is based on HeLa cells expressing the DNA binding domain (DBD) of the yeast transcription factor GAL4 fused to the ligand binding domain (LBD) of human hPPARγ along with the responsive element of GAL4 fused to luciferase was developed. This allows for transactivation assessment of activation of hPPARγ measuring the luciferase activation, enabling identification of PPARγ agonists and antagonists.

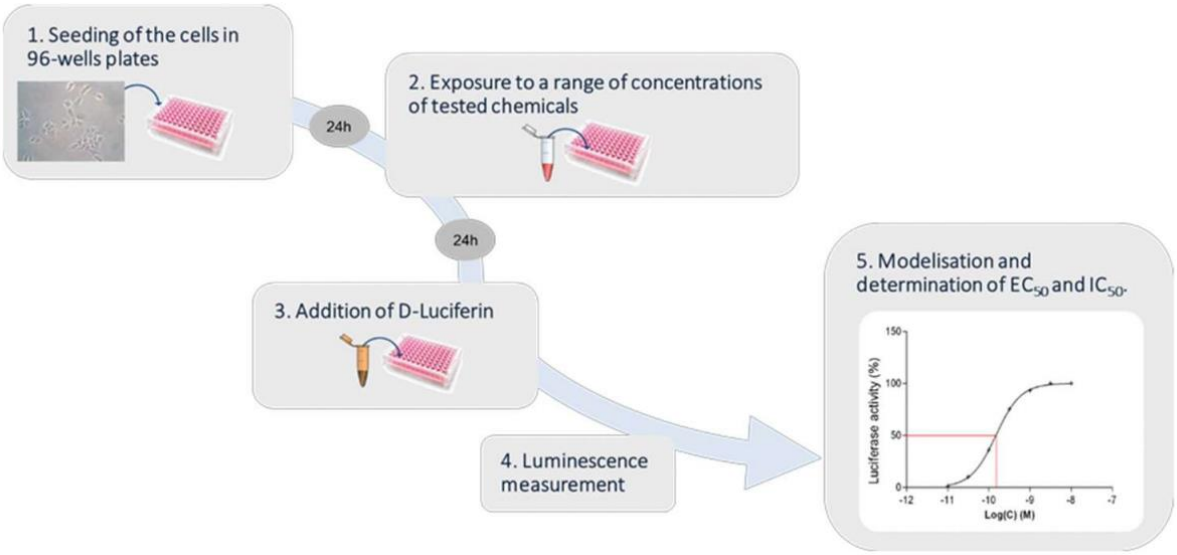

Figure 1: Workflow of the hPPARTA*. After seeding of the cells in 96-well plates, they are treated with a test compound for 24 hours. Thereafter, D-Luciferin is added, and Luminescence is measured.*

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.