Assessment of retinoic acid receptor (RAR)- and glucocorticoid receptor (GR)-dependent human neural progenitor cell proliferation arrest (NPC1_RAR_GR)

References:

Klose, J., Pahl, M., Bartmann, K., Bendt, F., Blum, J., Dolde, X., et al. (2021). Neurodevelopmental toxicity assessment of flame retardants using a human DNT in vitro testing battery. Cell Biol. Toxicol., 1–27. doi:10.1007/s10565-021-09603-2.

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Baumann, J., Gassmann, K., Masjosthusmann, S., DeBoer, D., Bendt, F., Giersiefer, S., et al. (2016). Comparative human and rat neurospheres reveal species differences in chemical effects on neurodevelopmental key events. Arch. Toxicol. 90, 1415–1427. doi:10.1007/S00204-015-1568-8.

Baumann, J., Dach, K., Barenys, M., Giersiefer, S., Goniwiecha, J., Lein, P. J., et al. (2015). "Application of the Neurosphere Assay for DNT Hazard Assessment: Challenges and Limitations," in Methods in Pharmacology and Toxicology (Totowa, NJ: Humana Press), 29. doi:10.1007/7653_2015_49.

Moors, M., Rockel, T. D., Abel, J., Cline, J. E., Gassmann, K., Schreiber, T., et al. (2009). Human Neurospheres as Three-Dimensional Cellular Systems for Developmental Neurotoxicity Testing. Environ. Health Perspect. 117, 1131–1138. doi:10.1289/EHP.0800207.

Summary of the method:

Foetal primary human neural progenitor cells (hNPC, gestational week 16-19) cultivated as three-dimensional floating spheres can represent several key processes during brain development. In the NPC1ab_RAR_GR proliferation assay, spheroidal hNPC are plated in 96-well plates and exposed to endogenous activators of the retinoic acid receptor (RAR) and glucocorticoid receptor (GR), i.e. all-trans retinoic acid and dexamethasone, respectively. Due to the anti-proliferative effects of both, RAR and GR activation as co-treatment with a test compound can identify inhibitors of the hormone receptors within a single assay setup. Moreover, putative agonistic effects of a test chemical can be identified by co-exposure to RAR and GR antagonists AL082D06 and mifepristone, respectively.

Readouts: sphere size increase (bright-field microscopy), BrdU incorporation into DNA(luminescence), mitochondrial activity and cytotoxicity (fluorescence)

The hNPCs used in this assay are purchased from Lonza (Lonza Verviers # PT-2599) and cultivated and passaged according to the scheme in figure 1.

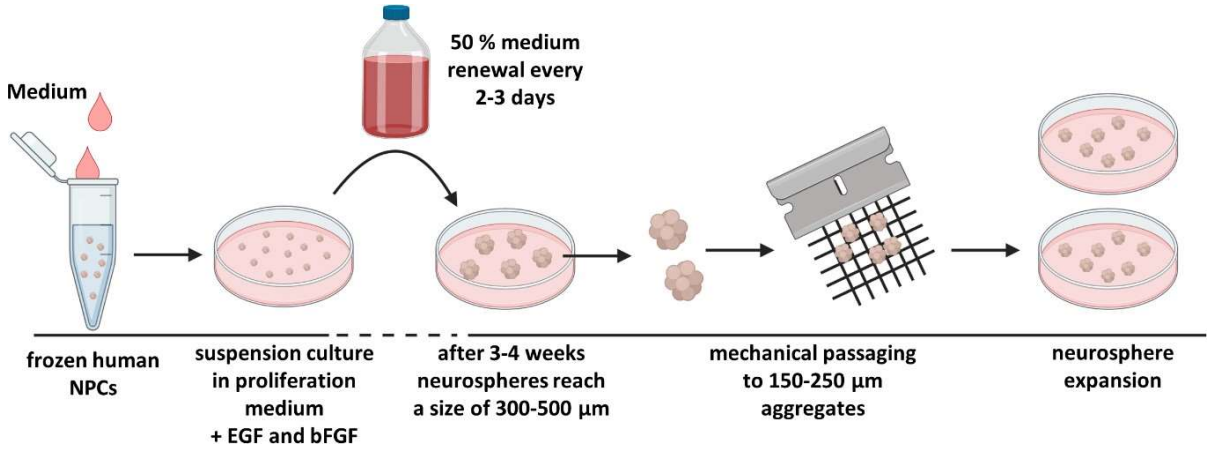
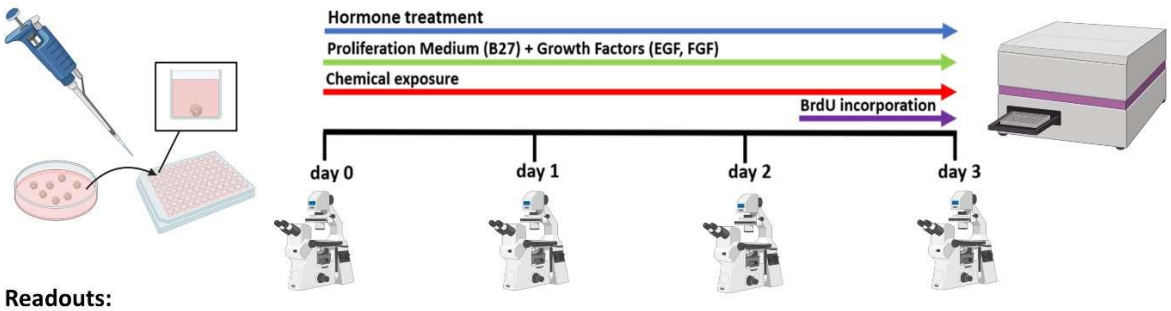


Figure 1 differentiation towards the final test system. *hNPCs are thawed by repeated addition and removal of proliferation medium. The resuspended cells are distributed to cell culture dishes and cultivated in proliferation medium with EGF and FGF for three to four weeks with 50 % media exchange every two to three days. When the spheres reach a size of 200 – 500 µm they are expanded by mechanical passaging every 5-7 days.*

Within the NPC1ab_RAR_GR assay chemicals are tested for either activation or inhibition of the RAR and GR. The workflow and the four readouts are depicted in figure 2.

Workflow:



Readouts:

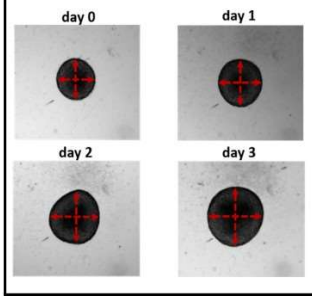
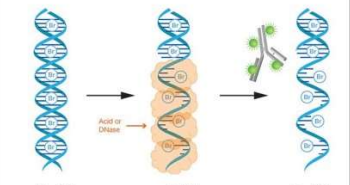
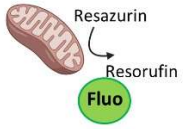
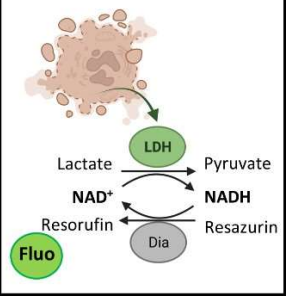
NPC1a: proliferation via sphere size increase	**NPC1b:** proliferation via BrdU incorporation into DNA	**Mitochondrial activity**	**Cytotoxicity**
			

Figure 2 Setup of the NPC1ab_RAR_GR assay. *hNPCs are mechanically passaged to 165 µm three days prior to the assay. On day zero they are plated one sphere per well into proliferation medium containing either hormone receptor agonists, antagonists and/or the test chemical and are cultivated for three days. Every day bright-field microscopy pictures are taken. 18 h prior to the end of the three-days period, BrdU labelling reagent is added. On day three, the mitochondrial activity of the cells is assessed with the Alamar blue method (Promega, #G8081), cytotoxicity is assessed by measuring lactate dehydrogenase (LDH) in the culture medium (Promega #G7890), and afterwards the spheres are dissociated and BrdU incorporation is quantified using the Cell Proliferation ELISA kit from Roche (Merck #11647229001). The sphere size is calculated from the bright-field images taken on day 0, 1, 2, and 3. Abbreviations: NPC, neural progenitor cells; BrdU, bromo deoxy uridine; Dia, diaphorase.*

Necessary equipment and consumables:

Experience with culture of neurospheres or other spheroid suspension cell cultures is advantageous!

- standard cell culture equipment (e.g. incubator, laminar flow hood)
- tissue chopper for mechanical passaging of cells (e.g. McIlwain Tissue Chopper, Campden Instruments)
- Cell Proliferation ELISA, BrdU (#11 669 915 001, Roche/Sigma, +2 to +8°C)
- CellTiter-Blue® Cell Viability Assay (e.g. #G8081, Promega)
- CytoTox-ONE™ Homogeneous Membrane Integrity Assay (#G7891, Promega), or equivalent
- Plate reader which can measure fluorescence (Extinction: 549 nm, Emission: 590 nm) and luminescence (e.g. Tecan Infinite M200Pro)
- Accutase (#A1110501; Life Technologies), or equivalent
- 96-well plates, clear, sterile, not TC-treated, U-bottom (e.g. #351177, Falcon)
- 96-well plates, white, pureGrade™, F-bottom (#781605, Brand)
- poly-(2-hydroxyethyl methacrylate) (e.g. #P3932, Sigma-Aldrich)

Proliferation medium:

- DMEM (e.g. #31966-021, Life Technologies)
- 33% Hams F12 (e.g. #31765-027, Life Technologies)
- 2% B27 (#17504044, Life Technologies)
- 20 ng/mL EGF (#PHG0313, Thermo Fisher)
- 20 ng/mL FGF basic (#233-FB, R&D Systems)
- 100U/mL penicillin and 100 µg/mL streptomycin (e.g.#P06-07100, Pan-Biotech)

Authorisation of use of cells may be required (depending on the country).