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CONCEPT LIFE SCIENCES

Concept Life Sciences: **Sodium/Iodide symporter (NIS) assay**

Introduction

The NIS assay measures the uptake of iodide into rat FRTL-5 cells in the presence and absence of test item.

The NIS is a co-transporter that mediates uptake of iodide into follicular cells of the thyroid gland as the first step in the synthesis of thyroid hormone. This assay is a spectrophotometric method run with a positive control (and negative control if requested by client). Cell viability is also checked to enable accurate interpretation of results.

Deliverable: Study report detailing protocol, data and IC50 of test compound.

Endocrine disruptor assessment

Regulators are concerned about the potential for environmental chemicals such as agrochemicals and their metabolites to perturb hormone systems. This has led to recommendations for the testing of potential endocrine disrupting chemicals.

Concept Life Sciences are experts in regulatory studies for investigative mechanistic toxicology. Our specialist team of scientists offer a suite of endocrine disruptor assays which can be used to compare results across species and interrogate different mechanisms of thyroid hormone modulation, to evaluate the relevance of in vivo toxicology findings.

- Sodium/Iodide symporter; NIS assay (rat)
- Deiodinase; DIO assay (DIO1, 2, and 3, rat, dog, human)
- Thyroperoxidase activity; TPO assay (rat, dog, pig, human)
- Thyroid hormone receptor assay
- Cross species comparative induction of UGT gene expression (rat, human)

Customer provides

Compound identifier, molecular formula and batch molecular weight. Test item: 200mg solid.

Cell line

FRTL-5 rat clonal thyroid follicular cell line cultured in defined conditions to retain thyroid-like features including expression and appropriate cellular localization of NIS, uptake of iodide and expression of thyroglobulin.

Test compound

Concentration range 0, 0.0005, 0.005, 0.05, 0.5, 5, 50, & 500µM or up to solubility limit.

Format

96-well plate, 200µL incubation volume. Data are reported for N = 4.

Controls

Assay acceptance criteria are available on request. Sodium perchlorate, a known NIS inhibitor, is selected as positive control (IC50 range 01- 0.9 µM). A negative control can also be added if requested.

Positive control	IC_{50} uM
Sodium Perchlorate	0.1–0.9 µM

Protocol summary

Plated FRTL-5 cells are incubated with test items and the known NIS inhibitor sodium perchlorate (positive control) for the defined incubation period; sodium fluoride can be included as a negative control (if requested by client). Cellular iodide accumulation is determined based on the catalytic effect of iodide on the reduction of yellow cerium(IV) to colorless cerium(III) in the presence of arsenious acid (Sandell-Kolthoff reaction)(Waltz et al 2010). The rate of reaction compared to control is proportional to the iodide concentration, and a reduction of activity indicates inhibition of iodide uptake by NIS. In addition, in a separate assay plate, FRTL-5 cell viability in the presence of test items and sodium perchlorate is determined by measuring ATP content.

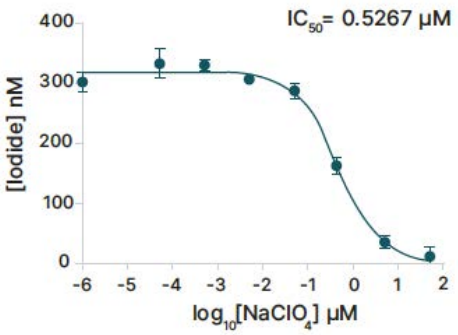
Quantitation

The supernatants are analyzed for absorbance at 420nM to quantitate uptake and luminescence to assess cell viability. Raw data is collated and analyzed using GraphPad Prism®.

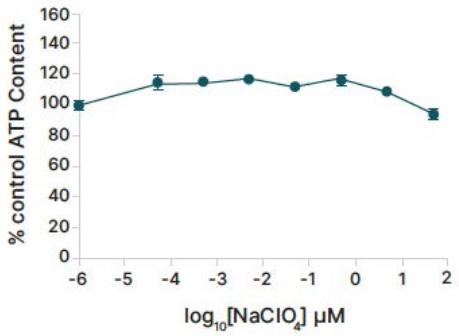
Sandell-Kolthoff reaction

$Ce^{4+} + 1/2\ As^{3+}$	$Ce^{3+} + 1/2\ As^{5+}$
Yellow	Colourless

NIS assay: sodium perchlorate



ATP assay: sodium perchlorate



Waltz F, Pillette L and Ambroise Y (2010). A nonradioactive iodide uptake assay for sodium iodide symporter function. Anal Biochem. 396 (1): 91-5