Neutral Red Uptake Assay

Commonly used acronym: *NRU*

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	C3a cells (Hepatocellular Carcinoma Cells, the C3A cell line is a clonal derivative of Hep G2 cells)

DESCRIPTION

Method keywords

neutral red
IC50
NI50
cell viability test

Scientific area keywords

in vitro toxicology
in vitro cytotoxicity
acute toxicity

Method description

The neutral red uptake (NRU) assay provides a quantitative measurement of the number of viable cells. The test is based on the ability of living cells to take up and bind neutral red (NR), a dye which easily penetrates cell membranes via non-ionic diffusion and accumulates in the lysosomes. Dying cells have altered membrane properties and therefore they cannot take up neutral red (NR) anymore. Consequently, living cells can be distinguished from dead or dying cells based on their different NR uptake. After the cells

have been exposed to the dye for three hours they are briefly washed with PBS. The incorporated dye is then liberated from the cells in an acidified ethanol solution. Released neutral red (proportional to the amount of Viable cells) is measured at OD 540 nm (OD 620nm as reference). Measured OD 540 nm of unexposed cells is set to 100% viability. Based on the absorption values, viability curves can be established to determine the concentration of test substance that is responsible for 50 % inhibition of NRU (IC50 or NI50).

Lab equipment

- Standard equipment for working with cell cultures ;
- Spectrofotometer.

Method status

History of use
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Very sensitive and readily quantifiable;
- Simple, fast, accurate and yields reproducible results;
- Cheap.

Challenges

- Crystal formation neutral red dye (critical step in the protocol);
- Possible Interference with colored compounds.

Future & Other applications

Use of the NRU assay with non-adherent cells (like TK6 cells).

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Borenfreund, E. and Puerner, J. [1985] Toxicity determined in vitro by morphological alterations and neutral red absorption. Toxicol. Lett. 24:119-124.
Repetto G, Del Peso A, Zurita JL. Neutral red uptake assayfor the estimation of cell viability/cytotoxicity. Nat Prot. 2008;3:1125–31.
Ndhlala AR, Anthonissen R, Stafford GI, Finnie JF, Verschaeve L, Van Staden J. In vitro cytotoxic and mutagenic evaluation of thirteen commercial herbal mixtures sold in KwaZulu-Natal, South Africa. S Afr J Bot. 2010;76:132–8.

Associated documents

NRU-revieuw.pdf
In vitro cytotoxic and mutagenic evaluation of thirteen commercial herbal mixtures sold in KwaZulu-Natal, South Africa.pdf
Toxicity determined in vitro by morphological alterations and neutral red absorption..pdf

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