Assaying Cellular Viability Using the Neutral Red Uptake Assay

Commonly used acronym: *NRU*
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SCOPE OF THE METHOD

Alternative method relates to	Human health
Alternative method is situated in	Basic Research, Education and training, Regulatory use - Routine production
Type of alternative method	In vitro - Ex vivo
This method makes use of	Human derived cells / tissues / organs
Specify the type of cells/tissues/organs	Hepatic cell lines such as HepG2, HepaRG. Other cell lines also possible, e.g. 3T3 mouse fibroblasts.

DESCRIPTION

Method keywords

cellviability

toxicity

acute toxicity

neutral red uptake

HepG2

Scientific area keywords

in vitro toxicity

viability study

hepatic toxicity
basal toxicity

Method desription

The neutral red uptake assay is a cell viability assay that allows in vitro quantification of xenobiotic-induced cytotoxicity. The assay relies on the ability of living cells to incorporate and bind neutral red, a weak cationic dye, in lysosomes. As such, cytotoxicity is expressed as a concentration-dependent reduction of the uptake of neutral red after exposure to the xenobiotic under investigation. The neutral red uptake assay is mainly used for hazard assessment in in vitro toxicology applications.

Lab equipment

Incubator (37 ± 1 °C, 90 ± 5% humidity, 5.0 ± 1% CO2/air)
Laminar flow / clean bench / cabinet (standard: “biological hazard”)
Water bath (37 ± 1 °C)
Inverse-phase contrast microscope
Laboratory balance
96-Well plate spectrophotometer (i.e., plate reader) equipped with 540 ± 10 nm filter
Shaker for microtiter plates
Cell counter or hemocytometer
Pipettes, pipettors (multichannel and single channel; multichannel repeater pipette)
96-Well flat-bottom tissue culture microtiter plates
Multichannel reagent reservoir
Vortex mixer

Method status

Published in peer reviewed journal
Validated by an external party (e.g. OECD, EURL ECVAM,...)

PROS, CONS & FUTURE POTENTIAL

Advantages

Fast
Accurate

Cheap

Challenges

Relatively easy to perform

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Ates, Gamze, Tamara Vanhaecke, Vera Rogiers, and Robim M. Rodrigues. "Assaying Cellular Viability Using the Neutral Red Uptake Assay." Cell Viability Assays: Methods and Protocols (2017): 19-26

Associated documents

NRU Book chapter.pdf

PARTNERS AND COLLABORATIONS

Organisation

Name of the organisation Vrije Universiteit Brussel

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Coordinated by

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