Assaying Cellular Viability Using the Neutral Red Uptake Assay

Commonly used acronym: *NRU*

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Organisation

Name of the organisation Vrije Universiteit Brussel (VUB)
Department Pharmaceutical and Pharmacological Sciences
Specific Research Group or Service In Vitro Toxicology and Dermato-Cosmetology
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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Education and training, Regulatory use - Routine production
Type of method	In vitro - Ex vivo
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 description

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

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