

## Assaying Cellular Viability Using the Neutral Red Uptake Assay

**Commonly used acronym:** NRU

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### Contact person

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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

**Country** Belgium

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research, Education and training, Regulatory use - Routine production
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	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 ( $37 \pm 1$  °C,  $90 \pm 5\%$  humidity,  $5.0 \pm 1\%$  CO<sub>2</sub>/air) ;  
Laminar flow / clean bench / cabinet (standard: “biological hazard”) ;  
Water bath ( $37 \pm 1$  °C) ;  
Inverse-phase contrast microscope ;  
Laboratory balance ;  
96-Well plate spectrophotometer (i.e., plate reader) equipped with  $540 \pm 10$  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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