

# Neutral Red Uptake Assay in C3a cells

*Commonly used acronym: NRU*

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## SCOPE OF THE METHOD

<b>Alternative method relates to</b>	Human health
<b>Alternative method is situated in</b>	Basic Research
<b>Type of alternative method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Human derived cells / tissues / organs
<b>Specify the type of cells/tissues/organs</b>	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 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. It accumulates in the lysosomes. Dying cells have altered membrane properties and therefore they cannot take up neutral red (NR) anymore. Living cells can therefore be distinguished from dead or dying cells based on their different NR uptake. After the cells have been allowed to incorporate 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. According to this, after exposure with test substance cell viability or viability curves can be established which enables determination of the concentration of test substance that is responsible for 50 % inhibition of NRU (IC50 or NI50).

### **Lab equipment**

Spectrofotometer

### **Method status**

History of use

### **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 assay for 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-revieww.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](#)

## PARTNERS AND COLLABORATIONS

### Organisation

**Name of the organisation** Sciensano

**Department** Chemical and physical health risks

**Specific Research Group or Service** Risk and health impact assessment

**Country** Belgium

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