# Neutral Red Cell Proliferation and Cytotoxicity Assay Kit

# Catalog number: AR1157

Boster's Neutral Red Cell Cell Proliferation Assay Kit provides a quantitative estimation of the number of viable cells in a culture using standard microplate absorbance readers.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

BOSTER
antibody and ELISA experts

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# Neutral Red Cell Proliferation and Cytotoxicity Assay Kit

**Catalog Number:** AR1157

## List of Components

| Description | Quantity | Volume | Contents | Catalog Number |
|---|---|---|---|---|
| Neutral Red Staining Solution | 1 | 10mL | 0.33% in DPBS | AR1157-1 |
| Neutral Red Solubilization Solution | 1 | 100mL | 1% Acetic acid , 50% Ethanol in water | AR1157-2 |

## Overview

| | |
|---|---|
| **Product Name** | Neutral Red Cell Proliferation and Cytotoxicity Assay Kit |
| **SKU/Catalog Number** | AR1157 |
| **Pack Size** | 1 kit (for 500 assays) |
| **Storage & Expiration** | Upon receipt store Neutral Red Cell Proliferation and Cytotoxicity Assay Kit at 4°C. It is stable at 4°C for one year. |
| **Equivalent** | Millipore Sigma (Product No. TOX4) |
| **For Use With (Equipment)** | Microplate Reader |
| **Detection Method** | Absorbance (540 nm) |
| **Format** | 96-well plate |
| **Label or Dye** | Neutral Red |
| **Sample Type** | Cell culture (adherent and nonadherent) |
| **Cite This Product** | Neutral Red Cell Proliferation Assay Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # AR1157) |
| **Application** | Measurement of cell proliferation in response to growth factors, cytokines and nutrients.<br>Measurement of cytotoxicity<br>To study cell activation<br>*Our Boster Guarantee covers the use of this product in the above tested applications. |

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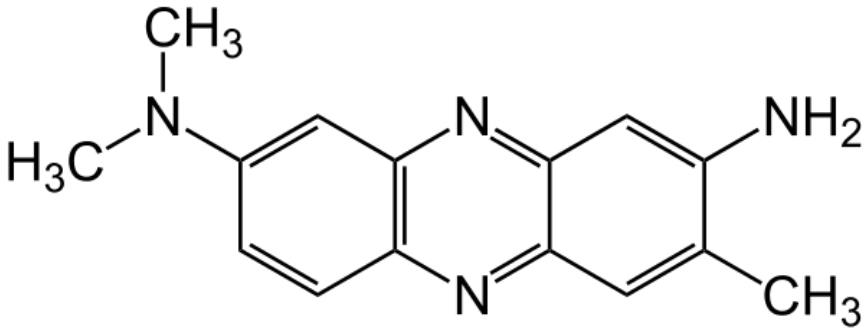
## Chemical structures

Molecular formula: C15H17N4
Molecular weight: 288.78
CAS number: 553-24-2




## Assay Principle

Neutral Red Cell Proliferation and Cytotoxicity Assay provides a quantitative estimation of the number of viable cells in a culture. This assay relies on the ability of neutral red to stain lysosomes of viable cells. Viable cells will take up the dye by active transport and incorporate the dye into their lysosomes, whereas non-viable cells will not take up the dye. After washing, viable cells can release the incorporated dye in an acidified ethanol solution. The amount of released dye can be used to determine the total number of viable cells or drug cytotoxicity. The neutral red uptake assay provides a quantitative measurement of the number of viable cells and can be measured at OD 540 nm. The result is a sensitive assay with excellent linearity up to approximately 10000 cells per well.

## Important Product Information

- The culture conditions used to grow the cells can affect the results and must be taken into consideration when analyzing the data. The age of the cultures, number of passages and details of the growth medium can all be important factors.
- For Neutral red staining solution, precipitates may form during storage, in which case filter the solution to remove the precipitates or pipette the supernatant of the solution for use.
- Uneven evaporation of culture fluid in wells of 96-well plate may cause erroneous result.
- Please wear gloves to operate.

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## Additional Materials Required

- 20μL, 200μL and multi-channel pipettes
- 96 well plate
- $CO_2$ incubator
- PBS
- Plate reader capable of reading absorbance at 540nm or spectrometer

## Assay Protocol

1. Add 200μL of the cell medium typically containing between 1000-10000 cells/well to a 96 well plate. And add compound.
2. Add 20 μL of Neutral Red Staining Solution to each well if there isn't any interference brought by the compound.
   **Note:** If the compound will interfere with the following assay, carefully remove the culture medium and washed the cells in an osmotically balanced saline solution such as PBS, DPBS or HBSS. Add 200μL of the cell medium to each well. And then add 20 μL of Neutral Red Staining Solution to each well.
3. Incubate the microplate at 37°C for 2 hours in a humidified chamber (5% $CO_2$). At low cell densities, the incubation time can be extended to 3-4 hours.
4. At the end of the incubation period, carefully remove the culture medium and washed the cells in an osmotically balanced saline solution such as PBS, DPBS or HBSS.
5. Add 200 μL of Neutral Red Solubilization Solution to each well. Gently stir in a shaker to enhance mixing of the solubilized dye.
6. Measure absorbance using a microplate reader or spectrometer at 540 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract from 540 nm measurement.