

Version 3b, Last updated 18 June 2025

ab234039

Neutral Red Assay Kit - Cell Viability / Cytotoxicity

For the measurement of cell viability or drug cytotoxicity in adherent and suspension cells.

This product is for research use only and is not intended for diagnostic use.

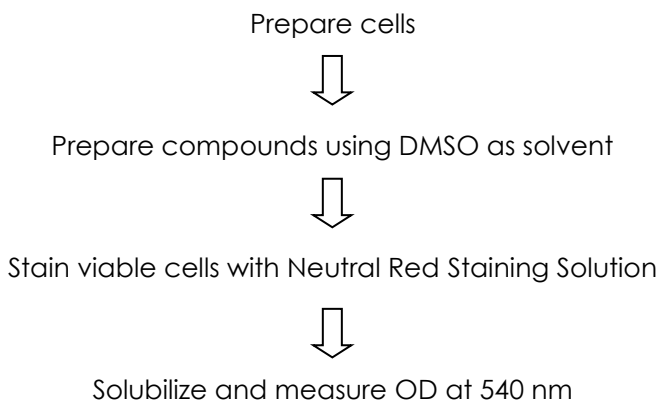
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1. Overview

Neutral Red Assay Kit - Cell Viability/Cytotoxicity (ab234039) is simple, accurate and reproducible. This kit offers an excellent and efficient method for *in vitro* cytotoxicity studies as well as high-throughput drug screening that can detect between 5,000-50,000 cells per well. It includes Doxorubicin as a positive control.

Neutral red cell cytotoxicity assay is one of the common methods used to detect cell viability or drug cytotoxicity. The principle of this assay is based on the detection of viable cells via the uptake of the dye neutral red. Neutral red is a eurythrin dye that stains lysosomes in viable cells. Viable cells can take up neutral red via active transport and incorporate the dye into their lysosomes but non-viable cells cannot take up this chromophore. Consequently, after washing, viable cells can release the incorporated dye in under acidified-extracted conditions. 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.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X Wash Buffer I	70 mL	-20°C	4°C
Doxorubicin	100 µL	-20°C	-20°C
2X Solubilization Solution I	75 mL	-20°C	4°C
100X Neutral Red Staining Solution	2 mL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 540 nm
- 96 well plate with clear flat bottom
- 100% methanol

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 10X Wash Buffer I

Prepare 1X Wash Buffer I by adding 1-part 10X Wash Buffer I to 9 parts dH₂O.

5.2 Doxorubicin

Ready to use as supplied.

5.3 2X Solubilization Solution I

Dilute 2X Solubilization Solution I 2-fold using 100% methanol to make 1X Solubilization Solution I.

Δ Note: *1X Solution is stable and can be stored at 4°C.*

5.4 100X Neutral Red Staining Solution

Prepare 1X Neutral Red Staining Solution by adding 1-part 100X Neutral Red Staining Solution to 99 parts cell culture medium.

Δ Note: *Do not store 1X Neutral Red Staining Solution. Discard unused Staining Solution if not used within 24 hours.*

6. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

6.1 Adherent cells:

1. Grow cells to ~80% confluency.
2. Trypsinize the cells and spin down the cells.
3. Remove the solution and add growing medium to disperse the cell pellet.
4. Determine the cell density by using a hemocytometer. Adjust the cell concentration if necessary.
5. Add 200 μL of the cells, typically containing between 5,000–20,000 cells/well, to a 96-well clear flat-bottom plate.
6. Incubate cells overnight in an incubator under standard cell conditions.

6.2 Suspension cells:

1. Spin down the cells.
2. Remove the culture media and add fresh growing media to adjust the cell density.
3. Add 200 μL of the cells, typically containing between 5,000–20,000 cells/well, to a 96-well clear flat-bottom plate.
4. Incubate cells overnight in an incubator under standard cell conditions.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all controls and samples in duplicate.

7.1 Compound Treatment:

1. Prepare compounds using DMSO as solvent.
2. Dilute compound stock solution in DMSO appropriately. Recommended final DMSO concentration in wells should be 0.5% or less.
3. Add diluted compounds to the wells.
4. Prepare a DMSO vehicle control and a background control (containing only the medium).
5. For inhibitor control; add 1 μL of doxorubicin to a well containing the cells.
6. Incubate the plate at 37 °C, in a 5% CO₂ controlled incubator for 72 hours.

7.2 Neutral Red Staining:

For adherent cells:

1. Remove the culture medium by gentle pipetting.
2. Add 200 μL of 1X Wash Buffer I/Washing Solution to wash wells. Washing should be done as gently as possible to avoid disturbance of the cell monolayer. Remove the wash solution as much as possible by pipetting.
3. Add 150 μL of 1X Neutral Red Staining Solution to each well and stain for 2 hours in an incubator.
4. After incubation, remove the staining solution.
5. Add 250 μL of 1X Wash Buffer I/Washing Solution to wash each well once. Remove the wash solution as much as possible by pipetting and air-dry the plate if necessary.

For suspension cells:

1. Spin down the cells and remove the medium carefully.
2. Add 200 μL of 1X Wash Buffer I/Washing Solution to wash wells.
3. Spin down the cells and remove the wash solution as much as possible.
4. Add 150 μL of 1X Neutral Red Staining Solution to each well and stain for 2 hours in an incubator.

5. After incubation, spin down the cells and remove the staining solution.
6. Add 250 μL of 1X Wash Buffer I/Washing Solution to wash each well once.
7. Spin down the cells and remove the solution as much as possible by pipetting and air-dry the plate if necessary.

7.3 Solubilization:

1. Add 150 μL of 1X Solubilization Solution I/Solubilization Solution to each well.
2. Shake the plate occasionally or place the plate on a shaker for 20 minutes at room temperature.

7.4 Measurement:

1. Measure the OD at 540 nm.

8. Data Analysis

Correct the background by subtracting the O.D. of the background control from all readings Calculate the percentage of cytotoxicity using the formula below:

$$\% \text{ Cytotoxicity} = \frac{O.D. \text{ DMSO} - O.D. \text{ sample}}{O.D. \text{ DMSO}} * 100\%$$

Where:

O.D. DMSO = is the O.D. of the DMSO control after background correction.

O.D. sample = is the O.D. of the sample after background correction.

9. Typical Data

Data provided for demonstration purposes only.

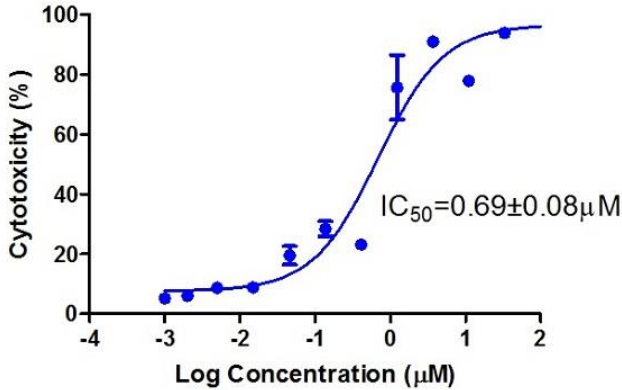


Figure 1. Dose-response curve of HepG2 (human liver hepatocellular carcinoma cell line) cells treated with doxorubicin for 72 hours determined using the Neutral Red Assay Kit - Cell Viability / Cytotoxicity (ab234039). Assays were performed according to the kit protocol in triplicate.

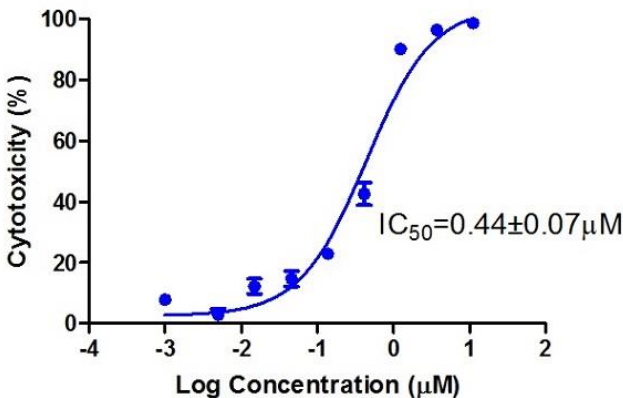


Figure 2. Dose-response curve of MCF7 (human breast adenocarcinoma cell line) cells treated with doxorubicin for 72 hours determined using the Neutral Red Assay Kit - Cell Viability / Cytotoxicity (ab234039). Assays were performed according to the kit protocol in triplicate.

10. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

11. Notes

Technical Support

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