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Neutral Red Cell Cytotoxicity Assay Kit

(Catalog # K447-1000; 1000 assays, Store kit at -20°C)

I. Introduction:

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 eurhodin 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 not 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. BioVision's Neutral Red Cell Cytotoxicity Assay Kit is simple, accurate, and reproducible. It also includes Doxorubicin, as a positive control. 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.

II. Application:

- *In vitro* cell proliferation cytotoxicity studies
- High-throughput drug screening

III. Sample Type:

- Cell culture: Adherent cells and non-adherent cells

IV. Kit Contents:

Components	K447-1000	Cap Code	Part Number
100X Neutral Red Staining Solution	2 ml	NM	K447-1000-1
10X Washing Solution	70 ml	NM	K447-1000-2
2X Solubilization Solution	75 ml	WM	K447-1000-3
20 mM Doxorubicin	100 µl	Red	K447-1000-4

V. User Supplied Reagents and Equipment:

- 96-well clear flat-bottom plate
- Multi-well spectrophotometer
- Personal Protective equipment: gloves, goggles and laboratory coat
- 100% Methanol

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. The kit components are stable for one year when stored as recommended. Neutral red dye is light-sensitive and should be protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Bring all reagents to room temperature (RT) before use.

- **10X Washing Solution**: Prepare 1X Washing Solution by adding 1 part 10X Washing Solution to 9 parts dH_2O. Store at 4°C.
- **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. **Note**: *Do not store 1X Neutral Red Staining Solution. Discard unused Staining Solution if not used within 24 hours*.
- **2X Solubilization Solution**: Dilute 2X Solubilization Solution 2-fold using 100% Methanol to make 1X Solubilization Solution. 1X Solution is stable and can be stored at 4°C.
- **20 mM Doxorubicin**: Ready to use. Store at -20°C.

VII. Neutral Red Cell Cytotoxicity Assay:

1. **Cell Culture**: Grow cells to ~80% confluency. For adherent cells, trypsinize the cells and spin down the cells. Remove the solution and add growing medium to disperse the cell pellet. Determine the cell density by using a hemocytometer. Adjust the cell concentration if necessary. Add 200 µl of the cells typically containing between 5,000–20,000 cells/well to a 96-well clear flat-bottom plate. Incubate cells overnight in an incubator under cell conditions. For suspension cells, spin down the cells. Remove the culture media and add fresh growing media to adjust the cell density. Add 200 µl of the cells typically containing between 5,000–20,000 cells/well to a 96-well clear flat-bottom plate. Incubate cells overnight in an incubator under cell conditions.
2. **Compound Treatment:** Prepare compounds using DMSO as solvent. Dilute compound stock solution in DMSO appropriately. Recommended final DMSO concentration in wells should be 0.5% or less. Add diluted compounds to the wells. Prepare a DMSO vehicle control and a background control (containing only the medium). For inhibitor control, add 1 µl of 20 mM doxorubicin to a well containing the cells. Incubate the plate at 37°C, 5% CO_2 controlled incubator for 72 hr.
3. **Neutral Red Staining**: For adherent cells, remove the culture medium by gentle pipetting. Fixation *(eg. by using 4% paraformaldehyde, not included)* may be required for low adherent cells). Add 200 µl of 1X Washing Solution to wash wells. *Washing should be done as gentle as possible to avoid disturbance of the cell monolayer*. Remove the wash solution as much as possible by pipetting. Add 150 µl of 1X Neutral Red Staining Solution to each well and stain for 2 hr in an incubator. After incubation, remove the staining solution. Add 250 µl of 1X 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: spin down the cells and remove the medium carefully. Add 200 µl of 1X Washing Solution to wash wells. Spin down the cells and remove the wash solution as much as possible. Add 150 µl of 1X Neutral Red Staining Solution to each well and stain for 2 hr in an incubator. After incubation, spin down the cells and remove the staining solution. Add 250 µl of 1X

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Washing Solution to wash each well once. Spin down the cells and remove the solution as much as possible by pipetting and air-dry the plate if necessary.

4. **Solubilization:** Add 150 µl of 1X Solubilization Solution to each well. Shake the plate occasionally or place the plate on a shaker for 20 min at RT.
5. **Measurement:** Measure the OD at 540 nm.
6. **Calculations:** Correct the background by subtracting the O.D. of the background control from all readings Calculate the percentage of cytotoxicity using the formula below:

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

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.

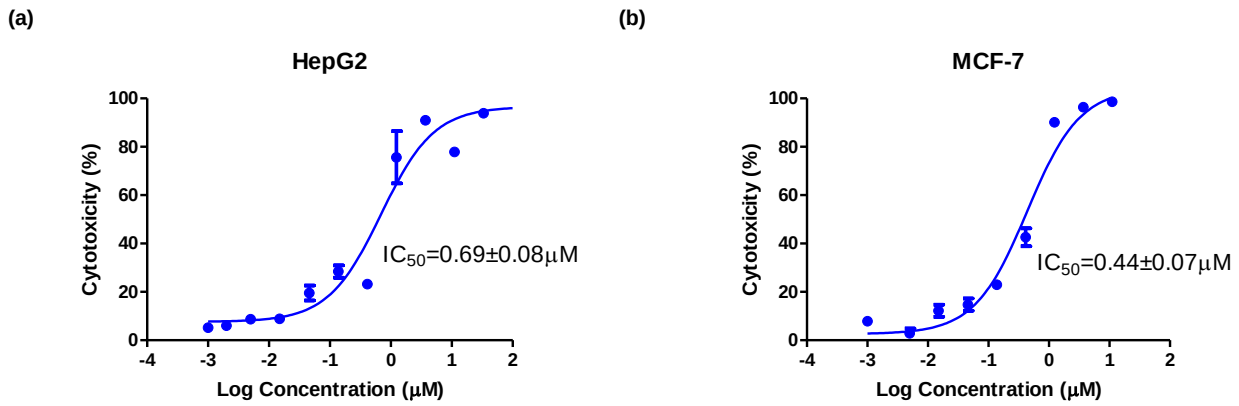

Figure: (a) Dose-response curve of HepG2 (a) and MCF-7 (b) cells to doxorubicin for 72 hr determined by the Neutral Red Cell Cytotoxicity assay. Assays were performed according to the kit protocol in triplicate.

VIII. RELATED PRODUCTS:

Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric) (K943)
LDH-Cytotoxicity Colorimetric Assay Kit II (K313)
Bioluminescence Cytotoxicity Assay Kit (K312)
PicoProbe™ LDH-Cytotoxicity Fluorometric Assay Kit (K314)
MTT Cell Proliferation Assay Kit (Colorimetric) (K299)
MTS Cell Proliferation Colorimetric Assay Kit (K301)
BrdU Cell Proliferation Assay Kit (K306)
StayBrite™ Highly Stable ATP Bioluminescence Assay kit (K791)
LDH-Cytotoxicity Colorimetric Assay Kit (K311)
ATP Colorimetric Assay Kit II (K354)
Senescence Detection Kit (K320)
PicoProbe™ Lactate Dehydrogenase Activity Assay Kit (K730)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)
ApoSENSOR™ ADP/ATP Ratio Bioluminescence Assay Kit (K255)

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