ab235935
Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric)

For the measurement of cytotoxicity in cultured adherent cells.

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

Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric) (ab235935) is simple, accurate, reproducible and sensitive. This kit
offers an excellent and efficient method for \textit{in vitro} cytotoxicity studies as well as high-throughput drug screening that can detect between 5,000-50,000 cells per well.

\begin{itemize}
  \item Grow cells to \sim 80\% confluency, trypsinize, wash and add 5,000-20,000 cells/well
  \item Treat cells with serial dilutions of test compounds
  \item Fix cells
  \item Stain cells with SRB Stain reagent
  \item Solubilize cells
  \item Measure absorbance at 565 nm
\end{itemize}

2. \textbf{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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature (before prep)</th>
<th>Storage temperature (after prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation Solution</td>
<td>55 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Washing Solution (20X)</td>
<td>50 mL</td>
<td>4°C or -20°C</td>
<td>4°C or -20°C</td>
</tr>
<tr>
<td>Solubilization Solution (10X)</td>
<td>22 mL</td>
<td>-20°C</td>
<td>4°C</td>
</tr>
<tr>
<td>SRB Dye Solution</td>
<td>50 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Doxorubicin (20 mM)</td>
<td>100 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
3. Materials Required, Not Supplied

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

- 96-well clear well plate
- Multi-well spectrophotometer capable of reading absorbance at 565 nm
- Personal Protective equipment: gloves, goggles, laboratory coat
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.

**Note:** Wear gloves and goggles when handling 20X washing and fixation solutions.

5.1 Washing Solution (20X)
1. Prepare 1X washing solution by adding 1 part of 20X washing solution to 19 parts distilled water.
2. You may need ~0.8 ml to wash each well.
3. 20X and 1X washing solutions can be stored at 4°C.

5.2 SRB Dye Solution
1. Ready to use as supplied.
2. Thaw SRB Dye Solution before use. Store at -20°C.

5.3 Solubilization Solution
1. Add 1 part of 10X Solubilization Solution to 9 parts distilled water.
2. Store at 4°C.

5.4 Doxorubicin (20 mM)
1. Ready to use as supplied.
2. Thaw doxorubicin before use. After use, doxorubicin should be stored at -20°C.

5.5 Fixation Solution
1. Ready to use as supplied.
2. Store at -20°C.
6. Assay Procedure

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

6.1 Cell culture:
1. Grow adherent cells to ~80% confluency.
2. Trypsinize and spin down the cells, add 5 ml of growth medium to disperse the cells.
3. Determine the cell density by using a hemocytometer. Add growth medium to the cells to adjust to an appropriate concentration.
4. Add 200 µL of the cells with a recommended density between 5,000–20,000 cells/well to a 96-well clear flat-bottom plate.

6.2 Compound treatment:
1. Prepare appropriate serial dilutions of your test compounds using DMSO as solvent.
2. Prepare a DMSO-only well as vehicle control, and another well containing culture medium-only as background control.
3. Add 1 µL of 20 mM doxorubicin to a well containing the cells as an inhibitor control.
4. Incubate the plate at 37°C in a humidified incubator with 5% CO₂ for 72 hours.

6.3 Cell fixation:
1. Without removing the culture medium, add ¼ volume (eg. 50 µl in 200 µL of culture medium) of the Fixation Solution to the each well. Incubate the plate for 1 hour at 4 °C.
2. Remove the solution and use 200 µL of dH₂O to wash the wells 3 times. **Washing should be done as gently as possible to avoid disturbance of the cell monolayer.** Remove wash solutions as much as possible by pipetting.
3. After cell fixation, washing and drying steps are complete, the plate can be stored at room temperature for a month if desired.

6.4 SRB Staining:
1. Add 45 µl of SRB Solution to each well and stain for 15 minutes at room temperature in the dark.

**Note:** SRB should be protected from light as it is light-sensitive.

2. After incubation, remove the staining solution. Add 200 µL of 1X Washing Solution to wash each well 4 times. Washing should be done as quickly as possible to avoid bleaching.

3. Remove wash solutions as much as possible by pipetting and air-dry the plate if necessary.

### 6.5 Solubilization:
1. Add 200 µL of 1X Solubilization Solution to each well.
2. Shake the plate occasionally or place the plate on a shaker for 10 minutes at room temperature.

### 6.6 Measurement:
1. Measure the absorbance at 565 nm.
2. If intense color is observed (> O.D. 3.5) due to cell overload you may use a suboptimal wavelength (eg. 490-530 nm) to lower the readings back to the linear range of your instrument.
7. Data Analysis

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (containing only culture medium) from all controls and sample readings. This is the corrected absorbance.
3. Calculate the percentage cytotoxicity as follows:

\[
\text{Cytotoxicity} \% = \frac{\text{O.D. (DMSO)} - \text{O.D. (Sample)}}{\text{O.D. (DMSO)}} \times 100\%
\]

Where:

- O.D. (DMSO) = O.D. of the DMSO control after background correction (corrected negative control well).
- O.D. (Sample) = O.D. of the sample after background correction.
8. FAQs / Troubleshooting

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

Data provided for demonstration purposes only.

**Figure 1.** Dose-response curve of HepG2 (a), MCF7 (b) and HEK-293 (c) cells, after exposure to doxorubicin for 72 hours, as determined using the Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric). Assays were performed according to the kit protocol in triplicate.
10. Notes
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