IN VITRO TOXICOLOGY ASSAY KIT
KENACID BLUE BASED

Stock No. TOX-5

Store at Room Temperature

IT IS RECOMMENDED THAT THE ENTIRE PROTOCOL BE REVIEWED BEFORE STARTING THE ASSAY.

Product Description
Traditionally, the in vitro determination of toxic effects of unknown compounds has been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The Kenacid Blue Assay System is a means of measuring total biomass via staining of cellular proteins by the dye Brilliant Blue R.

The Kenacid Blue Assay, as originally developed by Knox et al., is simple, accurate and yields reproducible results. The key component is the dye, Brilliant Blue R (identified as kenacid blue R in the original citation). The cells are briefly washed, fixed and stained with the dye. The incorporated dye is then liberated from the cells in an extraction solution. An increase or decrease in the number of cells (total biomass) results in a concomitant change in the amount of dye incorporated by the cells in the culture. This indicates the degree of cytotoxicity caused by the test material.

REAGENT
For Research Use Only.
Not for Use in Diagnostic Procedures.

Kit Components

<table>
<thead>
<tr>
<th>Product No.</th>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K3880</td>
<td>Kenacid Blue Assay Stain Solution (0.04% Brilliant Blue-R)</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

WARNING: Components in this kit should be carefully handled when using. Glutaraldehyde may be harmful if swallowed, inhaled, or absorbed through skin.

Product Storage
Kit components should be stored at room temperature.

Procedure
The kenacid blue method of monitoring in vitro cytotoxicity is well suited for use with multiwell plates. For best results, cells in the log phase of growth should be employed and final cell number should not exceed 10⁶ cells/cm². Each test should include a blank containing complete medium without cells.

1. Remove cultures from incubator into laminar flow hood or other sterile work area.
2. Remove medium and wash cells 3 times with 1X DPBS. 1X DPBS is prepared by diluting 10 ml of 10X DPBS [D1283] into 90 ml of sterile water.
3. Prepare fixative (3% glutaraldehyde) by diluting each bottle of 25% glutaraldehyde [G6257] with 70 ml of 1X DPBS prepared above. Fix the cells by gently removing DPBS and replacing with a volume of fixative equal to the original volume of culture medium.
4. Incubate plate for 10-20 minutes at room temperature. Blank background optical density is measured in wells incubated with growth medium without cells.

For additional technical information visit our website at http://www.sigma-sial.com

Sigma is a Member of the Sigma-Aldrich Family
Providing Biochemicals and Reagents for Life Science Research.
5. Add Kenacid Blue Stain Solution [K3880] in an amount sufficient to cover the culture surface area (approximately 50% of the culture medium volume).
6. Allow cells to stain for 20-30 minutes. Prepare wash solution by adding 50 ml of 5X wash solution to 200 ml of water.
7. At the end of the staining period, the stain is removed and the cells rinsed several times with the wash solution prepared in step 6.
8. The incorporated dye is then solubilized in a volume of Kenacid Blue Assay Extraction Solution [K3755] equal to the original volume of culture medium. The cultures are allowed to stand for 10-15 minutes at room temperature. Gentle stirring in a gyratory shaker or pipetting up and down (trituration) will enhance mixing of the solubilized dye.
9. Spectrophotometrically measure absorbance at a wavelength of 570 nm. If intense color is observed, a suboptimal wavelength (490-530 nm) can be used to facilitate reading of wells. Measure the background absorbance of multiwell plates at 690 nm and subtract from primary wavelength measurement.

Tests performed in multiwell plates can be read using an appropriate type of plate reader or the contents of individual wells may be transferred to appropriate size cuvets for spectrophotometric measurement.

Possible Sources of Error
1. Kenacid Blue may precipitate in solution upon storage. If precipitated dye crystals interfere with the assay, the dye solution may be filtered using a syringe filter before adding to the cell cultures.
2. Prolonged exposure of the cells to the wash solution can result in leaching of the dye into the wash solution.

References

For additional technical information visit our website at http://www.sigma.sial.com

Sigma is a Member of the Sigma-Aldrich Family
Providing Biochemicals and Reagents for Life Science Research.