XTT AND MTT CELL PROLIFERATION ASSAY KITS

Tetrazolium dye-based kits offer fast, accurate and proven methods to examine cell proliferation, cytotoxicity and apoptosis.

The measurement of cell viability and growth is a valuable tool in a wide range of research areas. Several approaches have been used in the past:

- Trypan blue
- Tritium-labeling
- Tetrazolium salts

The reduction of tetrazolium salts is now recognized as a safe, accurate alternative to radiometric testing. Tetrazolium salts are reduced in metabolically active cells to produce formazan. The color can then be quantified by spectrophotometric means. For each cell type a linear relationship between cell number and absorbance is established, enabling accurate, straightforward quantification of changes in viable cell number.

Both XTT and MTT tetrazolium salts have proven to be reliable and convenient quantitative, colorimetric methods for evaluating a cell population’s response to external factors, whether it be an increase in cell growth, no effect, or a decrease in growth due to necrosis or apoptosis. ATCC offers both methods for your convenience and preference.

### Kit Comparison

<table>
<thead>
<tr>
<th></th>
<th>XTT Kit</th>
<th>MTT Kit</th>
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</thead>
<tbody>
<tr>
<td>Detergent step</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Assay time (hours)</td>
<td>2 to 4</td>
<td>4 to 6</td>
</tr>
<tr>
<td>Assay type</td>
<td>Homogenous assay</td>
<td>End-point assay</td>
</tr>
<tr>
<td>Assays/kit</td>
<td>1,000</td>
<td>2,500</td>
</tr>
<tr>
<td>Cost/assay</td>
<td>$0.15</td>
<td>$0.09</td>
</tr>
</tbody>
</table>

**XTT CELL PROLIFERATION ASSAY KIT**

The ATCC® XTT Cell Proliferation Assay Kit incorporates a second generation tetrazolium salt to help you measure cell proliferation, cytotoxicity, and apoptosis in a fast, simple, high-throughput assay format. The assay kit provides:

- Fast results — an automation-friendly kit, geared for 96-well plates
- A simple one-step assay protocol — no solubilization required
- High sensitivity — homogenous assay using low cell numbers
- Reproducible accuracy — read-out is proportional to number of viable cells in exponential growth phase
- Non-radioactive — eliminates radioactive biohazardous waste
- Convenient storage — stable for 12 months when stored at -20°C in the dark

In cell-based assays, the second generation tetrazolium dye, XTT (sodium 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium inner salt), is reduced to a soluble, brightly colored orange derivative by a mix of cellular effectors. The sensitivity of an XTT assay is greatly improved by the usage of an intermediate electron carrier, PMS (N-methyl dibenzopyrazine methyl sulfate). PMS helps drive XTT reduction and the formation of its formazan derivative.
MTT CELL PROLIFERATION ASSAY KIT

The ATCC® MTT Cell Proliferation Assay Kit incorporates a tetrazolium salt to help you measure cell proliferation, cytotoxicity, and apoptosis in a convenient assay format. The MTT method offers several features for studying cell proliferation:

- Proven technology — documented in the literature for many different applications
- Accurate measurements — detect slight changes in cell metabolism, making it much more sensitive than trypan blue staining
- Safe reagents — no need to store or manipulate radioactive substances
- Easy to use — relatively simple and uses equipment already available in most labs
- Rapid processing — run in a 96-well plate and read with a microwell plate reader, allowing high-throughput handling of samples
- Convenient storage — stable for 18 months when stored at 4°C to 8°C in the dark

The ATCC MTT Cell Proliferation Assay is a colorimetric assay system which measures the reduction of the tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent for approximately 2 to 4 hours, a detergent solution is added to lyse the cells and solubilize the colored crystals. The samples are read using an ELISA plate reader at a wavelength of 570 nm. The amount of color produced is directly proportional to the number of viable cells.

The mouse fibroblast cell line L-929 (ATCC® CCL-1), was cultured overnight at 37°C in a 5% CO₂ incubator. No additional agents were added during the cell culture. Cell proliferation and viability of L-929 cells were assessed by XTT and MTT assays (3 hr assay incubation time). The XTT assay (measured at 475 nm) exhibits a wider dynamic range than the MTT assay (measured at 570nm) but showed a similar loss of linearity when greater than 2.5 x 10⁴ L-929 cells were used in the assays.

**Assessment of L-929 Cellular Proliferation by XTT and MTT Assays**

![Graph showing the comparison of XTT and MTT assay absorbance against cell number per well.]

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-1011K</td>
<td>XTT Cell Proliferation Assay Kit</td>
<td>1,000 assays</td>
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<tr>
<td>30-1010K</td>
<td>MTT Cell Proliferation Assay Kit</td>
<td>2,500 assays</td>
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