**MTT Cell Viability Assay**  
Colorimetric tetrazolium assay

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Amount</th>
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<tbody>
<tr>
<td>CPP-K03</td>
<td>25 assays</td>
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</table>

*For in vitro use only!*

**Shipping:** shipped at ambient temperature  
**Storage Conditions:** store at ambient temperature  
**Shelf Life:** 12 months

**Description:**  
MTT Cell Viability Assay Kit provides a highly sensitive, rapid, and easy-to-use tool for detection of cytotoxic agents present in cell cultures. The assay is based on life cell mediated reduction of MTT to a colored formazan derivative. Formazan formation is monitored at 508, 540, or 620 nm using a UV-VIS spectrophotometer or multiplate reader. Cell viability is determined in percent by comparison to control cell cultures. **MTT Cell Viability Assay Kit is especially designed for evaluating the effects of bioactive compounds such as cell penetrating peptides (CPPs) on viable cells.**

**Kit contents:**  
**MTT:** 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium-bromid  
25 mg  
**MTT stock solution**  
Dissolve MTT in sample buffer (25 mg in 5 ml, yielding a 5 mg/ml 10x stock solution). Final concentration in incubation medium is 0.5 mg/ml. The stock solution should be stored at -20 °C.

**Sample Buffer**  
200 ml  
1 N HCl  
0.5 ml  
DMSO  
50 ml  
Acidify DMSO by adding 1 % of 1 N HCl to DMSO immediately prior to use.

**Additionally required material**  
micropipettes  
centrifuge  
multiwell plate reader or spectrophotometer

**Protocol:**  
The protocol is calculated for 6-well / 35 mm culture plates or 2 ml tubes. For other vessels please adjust volumes of media accordingly:
- Cultivate cells at standard conditions  
- Prepare adherent or suspension cells according to general manual for Cell Penetrating Peptides, chapter 2.2.2 (Preparation of cells). Use 0.3 x 106 adherent cells per well or 1.0 x 106 suspension cells per 2 ml tube.  
- Aspirate cell culture medium thoroughly and wash cells 3x with 2 ml sample buffer at 37 °C.  
- Spike sample buffer (200 µl) with compounds to be evaluated [such as CPPs]. We recommend screening a range of concentrations. Use non-spiked sample buffer as control.  
- Add to cells and immediately add additional 400 µl of serum free medium.
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- Mix gently and incubate 1 h at 37 °C in a humidified chamber containing 5 % CO₂.
- Aspirate medium from cells.
- Mix 0.2 ml of MTT stock and 1.8 ml of complete growth medium and add to cells.
- Incubate 4 h at 37 °C in a humidified chamber containing 5 % CO₂.
- Remove about 85 % of medium from each well.
- Freshly prepare acidic DMSO and add to cells.
- Incubate for 10 min at 37 °C.
- Centrifuge for 5 min at 14,000 g and take off supernatant.
- Measure absorbance of supernatant at 508, 540 or at 620 nm.
- Compare to untreated cells (= 100 %).

Selected References:
Mussbach et al. (2011) Internalization of nucleoside phosphates into live cells by complex formation with different CPPs and JBS-Nucleoducin. Methods in Molecular Biology 683:375.

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