



ISO 13485:2016 ISO 9001:2015

Ver.P260901

MTT Cell Proliferation and Cytotoxicity Assay Kit

M1020 (500T)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

MTT can be reduced by dehydrogenases in mitochondria to produce dark-purple crystalline formazan. Formazan can be fully dissolved with the aid of specific solvent. Afterwards, the absorbance is measured by a microplate reader at approximately 490nm. Higher cell proliferation corresponds to higher absorbance, whereas stronger cytotoxicity results in lower absorbance.

Kit components

Reagent	500T
MTT Solution	5mL
Formazan Dissolving Solution	60mL

Protocol (only for reference)

1. Collect cells in logarithmic growth phase, adjust the cell suspension concentration, and dispense into a 96-well plate at 180µL per well (3000-10000 cells per well).
2. Incubate in a 37°C, 5% CO₂ incubator for 6-24 hours to allow cell attachment.
3. Add test compounds at appropriate concentrations and incubate for a suitable period of time.
4. Carefully remove the supernatant, add 90µL of fresh culture medium, followed by 10µL MTT solution, and continue incubation for 4 hours.
5. Remove the supernatant, add 110µL formazan dissolving solution to each well, and shake on a low-speed shaker for 10 minutes to fully dissolve the crystals.
6. Wrap the plate in aluminum foil or keep it in darkness to prevent formazan degradation. Shake the plate gently (100-150 rpm) for 10-15 min, and pipette stubborn crystals to ensure uniform dissolution.
7. Measure the absorbance of each well at 490nm using a microplate reader.
8. Blank wells: Wells filled with cell culture medium and the MTT reagent but not containing cells are known as blank wells. They are used to assess background absorbance from the medium or non-specific MTT reduction. Subtracting the absorbance of blank wells isolates the formazan signal, providing precise results.
9. Untreated control wells (negative control): These wells are filled with cells grown in regular conditions without any treatment. They depict basal metabolic activity and are deemed to be 100% viable.

Cell Viability (%) =

$$\left(\frac{\text{Absorbance of treated wells} - \text{Average absorbance of blank wells}}{\text{Average absorbance of untreated control wells} - \text{Average absorbance of blank wells}} \right) \times 100$$

Notes

1. Prevent evaporation by discarding the outermost wells and fill them with PBS, water or culture medium. Alternatively, place the 96-well plate close to the water source inside the incubator to mitigate evaporation.
2. MTT solution is yellow and should be stored protected from light; prolonged light exposure will cause inactivation. Do not use when the colour turns gray-green. If formazan dissolving solution freezes or forms precipitates, incubate in a 37°C water-bath to promote dissolution. Use only after complete dissolution and thorough mixing.
3. The products are all for scientific research use only. Do not use it for medical, clinical diagnosis.