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MTT assay

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We use this protocol and it's working

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Abstract

MTT assay was performed to investigate the cytotoxicity of samples against normal cells and leukemic cell lines according to these methods of Rueankham et al., 2023 and Moschini et al.,2023. The MTT dye is converted by NAD(P)H-dependent cellular oxidoreductase from viable mitochondria into insoluble formazan crystals.

Materials

Materials
1. PBS, pH 7.4
2. Histopaque-1077
3. RPMI-1640 containing 10% fetal calf serum, 1 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin
4. IMDM supplemented with 20% FBS, 100 units/mL penicillin and 100 µg/mL streptomycin
5. MTT dye solution
6. DMSO

Equipment
1. A humidified incubator
2. A centrifuge
3. A microplate reader
4. A laminar flow cabinet

MTT assay

1 100 µL of cell suspensions at the following cell concentrations, 2.5×105 cells/mL for PBMC, 1.5×104 cells/mL for KG1a, and 1.0×104 cells/mL for K562 or Molt4 were pipetted into the well
of the flat-bottomed 96-well plates.

2 100 µL of each sample at concentrations ranging from 3.125 to 200 µg/mL and doxorubicin (concentrations ranging from 0.01 to 4 µg/mL) as a positive control were added to cells. A medium
without a sample was used as a negative control.

3 incubated for 48 h at 37°C

4 15 µL of 0.2 mg/mL MTT dye solution was pipetted into each well and the plate was further incubated for 4 h at 37°C.

5 After the supernatants of the well were removed, the formazan crystals were dissolved in 200 µL of DMSO.

6 The absorbance of the solutions was measured at 578 nm using 630 nm as reference wavelength.

Protocol references

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