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## MTT (Assay protocol)

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

MTT ((**3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide**)) is used to assess cell viability as a function of redox potential. Actively respiring cells convert the water-soluble MTT to an insoluble purple formazan.

**AMJ13: 2 (Ahmed.Murtuda ,Jabria 2013)cell line:**

The cell line of breast cancer has been obtained from Iraqi breast cancer which originated from the prime tumor of an old Iraqi woman (70 years) with a histological identification with carcinoma of infiltrating ductal (1).

**SK-GT-4:**esophageal carcinomacell line was established from a primary tumors in 1989 from a 89 year-old Caucasian male who presented with dysphagia secondary to a well-differentiated adenocarcinoma arising in the Barrett epithelium of the distal oesophagus

## Materials

No	Item	Company	Country
1	incubator	Cypress Diagnostics	Belgium
2	Microtiter reader	Gennex Lab	USA
3	Laminar flow hood	K & K Scientific Supplier	Korea
4	Micropipette	Cypress Diagnostics	Belgium
5	Cell culture plates	Santa Cruz Biotechnology	USA

No	Items	Company	Country
1	Trypsin/EDTA	Capricorn	Germany
2	DMSO	Santacruz Biotechnology	USA
3	RPMI 1640	Capricorn	Germany
4	MTT stain	Bio-World	USA
5	Fetal bovine serum	Capricorn	Germany

## Maintenance of Cell lines

- 1 SK-GT-4 cell line, was maintained in MEM supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C
- 2 NHF cell line, was maintained in MEM supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C

## MTT Assay

- 3 To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates
- 4 Cell lines were seeded at  $1 \times 10^4$  cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compound
- 5 Cell viability was measured after 72h of treatment by removing the medium
- 6 adding 28 µL of 2 mg/mL solution of MTT
- 7 incubating the cells for 1.5 h at 37 °C.
- 8 After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide)
- 9 followed by 37 °C incubation for 15 min with shaking (orbital shaker)
- 10 The absorbency was determined on a microplate reader at 492 nm (test wavelength)
- 11 The assay was performed in triplicate

- 12 The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation

$$\% \text{ Cell viability} = \left( \frac{\text{Absorbance of treated cell}}{\text{Absorbance of non-treated cell}} \right) \times 100$$

$$\% \text{ Cytotoxicity} = 100 - \text{cell viability}$$

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