MTT (Assay protocol

Abdulkareem Hameed Abd[1], Enas Jawad Kadhim[2], Matin Mahmood[3]

[1]Department of Pharmacology, College of Medicine, Al-Nahrain University, Kadhimiya, Baghdad, Iraq;
[2]3 Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq;
[3]Department of Pharmacology, College of Pharmacy, Alkitab University, Altun Kopre, Kirkuk, Iraq

Matin Mahmood
Department of Pharmacology, College of Pharmacy, Alkitab Uni...

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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

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 1×10^4cells/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

% Cell viability = (Absorbance of treated cell / Absorbance of non-treated cell) x 100

% Cytotoxicity = 100 – cell viability