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Determination of viable cells by XTT

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol describes the test method for analysis of metabolic activity in various cell lines with colourimetric XTT analysis. It can be used to determine cytotoxicity of various drug compounds.

XTT salt (2, 3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) is a compound that is metabiolized by the purple dye formazan, in living cells, to form an orange colour. The colour change will only occur in the presence of these reagents and in living cells, so the amount of colour change can be used to quanitfy the amount of viable cells. The Beer-Lambert Law states that absorbance of a given wavelength will be proportional to concentration, and therefore the concentration of colour that is absorbed will be proportional to the number of viable cells.

PROTOCOL CITATION

tzhang 2021. Determination of viable cells by XTT. **protocols.io** https://protocols.io/view/determination-of-viable-cells-by-xtt-btisnkee

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GUIDELINES

1. Analyst is responsible for proper operation of instruments, proper documentation procedures, and proper reporting of assay results.
2. Laboratory Manager (LM) is responsible for coordinating containment and disposal of biohazardous materials including acquisition of proper documentation of disposal.
3. LM is responsible for reporting leakage or spills in the lab to proper authority.

MATERIALS TEXT

Materials:

XTT Sodium **Gold**

1. **Biotechnology Catalog #X-200-100**

Phosphate Buffered Saline **Gold**

2. **Biotechnology Catalog #P-271-50**

Phenazine Methosulfate **Millipore**

3. **Sigma Catalog #P9625-500MG**

Equipment:

1. ThermoFisher, Varioskan Lux Microplate Reader
2. Micro- Well plate
3. Micropipette and Tips.

SAFETY WARNINGS

Appropriate PPE must be used by all anlysts performing this experiment.
Consult SDS for all materials.
Operation of instrumentation should be conducted by trained employees of Sinoveda Canada Inc. Trainees should be supervised while operating equipment.
Hazardous materials should be handled according to their hazard class and must be identified by a workplace label.
Proper disposal of biohazardous materials should be followed.

1 Adhere cells to wells

1.1 Add a specified amount of cells from stock to each well of the micro-well plate. Each well must have a consistent cell density.
1.2 Incubate this plate at 37°C and 5% CO_2. Allow cells to adhere to the well plate for 24 hours.
1.3 Always include a control of untreated cells and a blank well with culture media with no cells.

2 Prepare the PMS and XTT solution

2.1 Prepare the MPS solution by dissolving 3 mg PMS (Phenazine Methosulfate) in 1mL of 1X PBS (Phosphate Buffered Saline).
2.2 Prepare the XTT solution by dissolving 4 mg of XTT in 4 mL of the cell culture media used.
2.3 XTT/PMS solutions can both be stored at **-20°C in the absence of light**. The expiry date will be **9 months from the date of preparation**. Discard if there is evidence of growth, colour change, or recyrstallization.

3 Cell labelling with XTT

3.1 Add 10 µL of the PMS solution to the 4 mL XTT solution to create the detection reagent.
3.2 Add 50 µL of the XTT/PMS solution to each of the wells.
3.3 Incubate the plate for 2-5 hours at 37 °C.

4 Sample Analysis

4.1 Place the reaction on a shaker for a short period to mix the dye and detection reagent.
4.2 Analyze the sample with a plate reader at λ= 450nm.