Determination of metabolic activity of cells by MTT test

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) 5 $g.l^{-1}$ in isotonic phosphate buffer pH 7.4 (**harmful to health**).

Extraction buffer (20% sodium lauroyl sulfate in 50% dimethylformamide, pH 4.7, **harmful to health**).

Principle

Yellow MTT is reduced by mitochondrial respiratory chain enzymes to a purple formazan derivative that remains inside the cells in the form of insoluble granules. After addition of detergent (sodium lauroyl sulfate, SDS) and acidification, the dye is released from the cells and dissolved, resulting in a clear solution suitable for photometric determination.

Procedure

1. Add 100 µl of MTT solution to 0.5 ml of mixed cell suspension and incubate for 45 minutes at 37 °C.
2. 0.5 ml of extraction buffer is added to the suspension, the mixture is mixed and incubated for 10 minutes at 37 °C.
3. Measure the absorbance at 595 nm against distilled water.

Links

Source

VEJRAŽKA, M.: Basic techniques of working with tissue cultures. Prague, 2004.