

MEDETOX

Innovative Methods of Monitoring of Diesel Engine Exhaust Toxicity in Real Urban Traffic (LIFE10 ENV/CZ/651)

PROTOCOL FOR PERFORMING A TRYPAN BLUE VIABILITY TEST

The dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, Eosin, or propidium, whereas dead cells do not. In this test, a cell suspension is simply mixed with dye and then visually examined to determine whether cells take up or exclude dye. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm.

Solutions

Phosphate-buffered saline (1x PBS)

NaCl	8 g
KCl	0.2 g
Na ₂ HPO ₄	1.42 g
KH ₂ PO ₄	0.24 g
pH	7.4
add dH ₂ O to	1 litre

Trypan blue

0.2% solution in PBS

Equipment

Microscope Olympus CKX41
Bürker counting chamber

Procedure

- 1) Mix 50 µl of the sample with 50 µl 0.2% solution of trypan blue in PBS.
- 2) Drop 50 µl of the suspension on a slide with a Bürker counting chamber.
- 3) Cover the slide with a coverslip and count cells under the microscope.
- 4) Determine number of viable cells (cells with a clear cytoplasm) and nonviable cells (cells with a blue cytoplasm).

CELL PROLIFERATION REAGENT WST-1 FOR THE QUANTIFICATION OF CELL PROLIFERATION, CELL VIABILITY, AND CYTOTOXICITY

The stable tetrazolium salt WST-1 is cleaved to a soluble formazan by a complex cellular mechanism that occurs primarily at the cell surface. This bioreduction is largely dependent on the glycolytic production of NAD(P)H in viable cells. Therefore, the amount of formazan dye formed directly correlates to the number of metabolically active cells in the culture. Cells, grown in a 96-well tissue culture plate, are incubated with the ready-to-use WST-1 reagent for 2 hours at 37°C. After this incubation period, the formazan dye formed is quantitated with a scanning multi-well spectrophotometer (ELISA reader). The measured absorbance at 440 nm directly correlates to the number of viable cells.

Chemicals and solutions

Cell proliferation reagent WST-1 (ROCHE Cat. No. 05015944001)

The unopened reagent is stable when stored at -15 to -25 °C, protected from light, through the expiration date printed on the label. Once thawed, store at +2 to +8 °C, protected from light, for up to four weeks. For longer storage keep in aliquots at -15 to -25 °C.

Etoposid ($M_r=588.6$)

Prepare the stock solution (20 mM) in DMSO: 11.8 mg/ml DMSO. For the cytotoxicity test use 2.5 µl, resp. 5 µl of the stock solution.

EOM (extractable organic mass) for an application

Dilute EOM to stock solution (10 µg/µl in DMSO), then dilute this stock solution in DMSO to work solution: 2.5 µg/µl, 1 µg/µl, 0.1 µg/µl.

For the cytotoxicity test use 10 µl of each work solution.

Equipment

Incubator Jouan IG150 (37 °C)
Centrifuge Eppendorf 5810 R
Spectrophotometer Spectra Max M5^e
Microscope Olympus CKX41
Bürker counting chamber
Multichannel pipette Eppendorf (10, 50, 100 µl)
96-well plates, sterile tips

Procedure

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1. Cells were seeded in a 96-well tissue culture plate in concentration 1×10^4 / well and grown for 1 day in 100 ul culture medium. The medium was then replaced with 100 ul fresh medium containing the test chemicals.
2. Pipette 10 μ l of WST-1 reagent per well.
3. Incubate cells for 2 h at 37 °C.
4. Shake thoroughly for 1 min on a shaker.
5. Measure the absorbance of the samples at 440 nm using a microplate reader. The reference wavelength should be more than 600 - WST-1 and formazan have the same absorbance in this area.