Cytotoxicity measurement in cultured primary rat hepatocytes

Commonly used acronym: *MTT assay*

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	rat
Type of cells/tissues/organs	primary hepatocytes

DESCRIPTION

Method keywords

cell viability test

MTT cytotoxicity assay

cytotoxicity of chemicals

mitochondria

Succinate dehydrogenase

Formazan

Scientific area keywords

Cell culture

cell viability

Primary hepatocytes

rat

liver

Method description

The MTT test is performed to determine the *in vitro* cytotoxicity of selected chemicals. The mitochondrial enzyme succinate deydrogenase is responsible for the biotransformation of toxic agents and MTT. The ability of cells to reduce MTT provides an indication of the mitochondrial integrity and activity, which in turn may be interpreted as a measure of viability and/or cell number. When chemical compounds are induced in primary rat hepatocytes, their cell viability and their possibility to transform xenobiotical substances decreases. In this respect, when the MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is added to the exposed cells, the possibility to transform this pale yellow salt into dark blue formazan crystals decreases. The formazan crystals formed in the cells are solubilized in DMSO and can be measured colorimetrically.

Lab equipment

Multiplate reader.

Method status

History of use

PROS, CONS & FUTURE POTENTIAL

Advantages

Easy to apply;
Not so many extra materials or solutions needed;
The method itself is very fast (4h).

Challenges

The density of the cells should be even in each well. Make sure that the cell suspension is homogenous and devoid large cell aggregates.
There are possible interferences between the tested chemical and the MTT as substrate. MTT can be directly reduced by test substances and give artifacts. Therefore, before initiating experiments, a special procedure that allows quantification of the ''true'' MTT mitochondrial reduction from the ''false'' chemical MTT reduction should be performed.
Considerable cell death is observed shortly after isolation of hepatocytes from a freshly removed liver and during the early phases of cultivation. To reduce the effects of this experimentally-induced cell injury and thus to avoid false positive results, experiments should be initiated at earliest 24h after cells seeding.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

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