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Nanotechnologies - In vitro MTS assay for measuring the cytotoxic effect of nanoparticles

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1.Structure and Drafting Rules of Standardization Documents"

drafted.

This document is equivalent to ISO 19007.2018 "Determination of cytotoxicity of nanoparticles by nanotechnology MTS method".

The following minimal editorial changes have been made to this document.

--- Modified the error numbers of the original texts B.2.2.3 and C.2.1.2.3 (see Appendix B and Appendix C);

--- Adjust some of the contents in C.2.1.2.1 and C.2.1.2.3.12 to "Note";

--- References reordered.

Please note that some content of this document may be patented. The issuing agency of this document assumes no responsibility for identifying patents.

Introduction

With the continuous development of new materials, products and applications, the field of nanotechnology continues to develop rapidly. At the same time, some of the

Concerns about potential risks to human health and the environment posed by rice-based materials are also on the rise. At present, a large number of related researches are being carried out internationally

research to better understand and quantify these potential risks. In addition, the surface coating of nanoparticles during processing or in the final product

Chemicals may also affect the interaction between nanoparticles and cells. Especially considering the large specific surface area of nanoparticles, this

The impact may be more pronounced.

Cells are the basic unit of life. Monitoring the biological response of model cells following nanoparticle exposure promises to deepen our understanding of the role of nanoparticles

patterns", and to determine which of these factors may require further study for subsequent risk assessment.

In.2008, some international research teams found that some published nanomaterial toxicity research results could not be replicated in different laboratories.

Therefore, there is a need to develop accurate and reproducible nanotoxicological testing methods. International Consortium for Nano Environmental Health and Safety (NanoEHS) Coordination

(IANH) came into being to develop test protocols that can accurately assess the toxicity of nanoparticles and their effects on cells, and these test methods

The case can be repeated in any laboratory. IANH has passed several common cytotoxicity tests to determine the particle size distribution in liquid suspension and nanoparticle size distribution.

The in vitro interaction of rice materials with cells was compared in experiments (Appendix A). The alliance identified a number of factors that would increase data volatility

elements, and developed techniques to reduce data volatility. National Institute of Environmental Health Sciences (NIEHS)

NanoGo)-funded research further evaluated some of the testing protocols, especially 3-(4,5-dimethyl-2-thiazolyl)-5-(3-carboxymethoxy

Detection scheme for phenyl)-2-(4-sulfophenyl)-2H-tetrazole (MTS) [4]. A third team extended IANH's approach and adopted a unified design

The multi-well plate of the meter was used for experiments, which improved the consistency of the analysis results (Appendix B) [5]. Of particular importance is the fact that the above projects are based on multiple real

Interlaboratory testing to identify sources of uncertainty and improve test methods.

This document uses the MTS method to assess the viability of cells in vitro [6]. The method utilizes the formazan (absorbing

The peak is located at 490 nm) to produce a color reaction. Typically, the change in absorbance is proportional to the number of cells. However, during the detection process

Changed reductase activity or insufficient reagents may also lead to color reaction, resulting in inaccuracy between absorbance and cell viability (such as cell number).

than relationship. Adding the MTS reagent directly to the cell culture plate allows a rapid

assessment of the potential endotoxicity of nanoparticles. due to nanoparticles

Particles may potentially interfere with colorimetric analysis, so before giving final test results, it is not advisable to conduct a control experiment of the interaction between the two.

very important. Direct observation of treated cells under the microscope is an orthogonal method for validating MTS assay results. The regulations set forth in this document

The normalized protocol is only used for adherent cells and can also be used for suspension cells after modification.

Toxicity testing of nanoparticle effects on individual cell lines in this method is a primary test. The standard approach presented in this document is based on the above

3 MTS analysis protocols. The differences between the experimental systems are shown in Table 1.

Table 1 Summary of studies used to develop standardized MTS assay protocols

Sponsoring Institution Cell Line a Nanoparticles for Testing b Positive and Negative Control Materials Are Centrifuged

IANH Raw264.7 PS-NP, CeO2 CdSO4, no particle exposure no

NanoGo BEAS-2B, RLE-6TN and THP-1 ZnO, TiO2, MWCNT No particle exposure Yes

EMPA-NISTc A549 PS-NP CdCl2, no particle exposure no

Name in the aATCC cell bank.

b PS-NP are positively charged PS nanoparticles, CeO2 is ceria, ZnO is zinc oxide, TiO2 is titanium dioxide, MWCNT is multi-walled carbon nanoparticle

meter tube.

cEMPA is the Swiss National Laboratory for Materials Science and Technology.

Because of these differences, optional steps proposed by 3 laboratory studies were included in the standard method.

In addition to the MTS method [6], there are other methods that can be used to measure cell viability, such as 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl bromide

Tetrazolium (MTT) method[7],
2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) method [8], lactate dehydrogenase

(LDH) method [9], trypan blue exclusion method [10] and neutral red assay [11]. The MTS method has carried out multiple comparison experiments. The MTS method is a modified

The advanced MTT method can provide a simple and high-throughput detection of cell viability[4,12]. After MTS is reduced by functional enzymes in living cells, the solution is

Optical density increases.

Control experiments are required to determine baseline optical densities of cell viability in untreated cells and to verify that cells respond to known nontoxic nanoparticles

particles, toxic chemicals, and toxic nanoparticles have expected responses [13]. In addition, determine whether nanoparticles interfere with the optical readout of the analysis and can

It is important to invalidate the assessment of nanoparticle cytotoxic responses [14].

Notably, the MTS method described here is one of many commercial methods used to assess the cytotoxicity of nanomaterials. Although this

The document does not address the LDH method for assessing plasma membrane integrity, the ATP method for assessing energy metabolism, and the BrdU method for DNA synthesis, etc.

Combined with the MTS method, the comprehensive effect of nanoparticles on cells can be more comprehensively evaluated.

Nanotechnology MTS method for determination of nanometers

Cytotoxicity of particles

1 Scope

This document describes the MTS method for evaluating the effect of nanoobjects and their aggregates and aggregates (NOAA) on cell viability. this

Methods include operational requirements and control experiments to determine and analyze the uncertainty of test results.

This document applies to 96-well plates.

2 Normative references

The contents of the following documents constitute essential provisions of this document through normative references in the text. Among them, dated citations

documents, only the version corresponding to that date applies to this document; for undated references, the latest edition (including all amendments) applies to

this document.

ISO /T S80004-2 Nanotechnology Terminology Part 2.Nano-objects (Nanotechnologies-Vocabulary-