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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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Nanotechnologies -- 5-(and 6)-Chloromethyl-2', 7' Dichloro-dihydrofluorescein diacetate (CM-H2DCF-DA) assay for evaluating nanoparticle-induced intracellular reactive oxygen species(ROS) production in RAW 264.7 macrophage cell line

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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 uses the translation method equivalent to ISO /T S19006:2016 "Nanotechnology Fluorescein Diacetate Method for the Detection of Nanoparticle Inducements"

Reactive Oxygen Species Produced by Macrophages":

The Chinese documents that have a consistent correspondence with the international documents normatively cited in this document are as follows:

---GB/T 16886:5-2017 Biological Evaluation of Medical Devices Part 5: In Vitro Cytotoxicity Test (ISO 10993-5:

2009, IDT):

The following editorial changes have been made to this document:

--- The documents cited in the introductory phrase of the Chapter Terms and Definitions are listed in Chapter 2 "Normative Reference Documents";

--- 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 coatings used to coat the surface 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: Therefore, it is necessary to develop reliable and rapid screening methods for these chemically functionalized nanoparticles:

Potential particle toxicity:

Monitoring the biological response of model cells after nanoparticle exposure promises to deepen our understanding of the nanoparticle "mode of action", and to judge

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 nanoparticle toxicity and its biological effects on cells, and these tests

The test procedure can be repeated in any laboratory: IANH adopts 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonic acid)

phenyl)-2H-tetrazole (MTS) method, 5-(6)-chloromethyl-2',7'-dichloro-dihydrofluorescein diacetate (CM-H2DCF-DA) method and Iodine

The propidium method was used to compare the particle size distribution in liquid suspension and the in vitro interaction of nanomaterials with cells: IANH Discovery

Some factors that increase test uncertainty have been identified, and techniques have been developed to reduce such uncertainty:

Oxidative stress can lead to DNA damage and is the main driving force for the accumulation of mutations in organisms: Therefore, it was assessed whether the nanoparticles would lead to

It is very important to activate cells to produce reactive oxygen species (ROS):

This document provides an in vitro measurement to assess ROS production from cellular exposure to nanoparticles: Although measuring intracellular ROS production has

There are many techniques, but only the CM-H2DCF-DA method has been carried out to evaluate the ROS production of RAW264:7 mouse macrophages:

The CM-H2DCF-DA method is a common method to detect cellular oxidative stress, which is not specific for oxygen free radicals or oxygen-reactive species [9]:

Although this method has not been evaluated in a wider range of cell lines, it provides insight into the possibility of ROS production in macrophages,

This mechanism may play an important role in clearing particles from the body:

In the presence of a control group, the CM-H2DCF-DA method may also be misjudged: Several factors may contribute to false negatives [9]:

The CM-H2DCF-DA method does not achieve optimal detection for all ROS: For example, for the short half-life superoxide anion and hydroxyl

Free radicals, its detection effect is not good: 2',7'-Dichlorodihydrofluorescein (DCFH) and 2',7'-Dichlorofluorescein (DCF) can be leached from cells

Or DCFH is oxidized, so when measured by flow cytometry, it should be detected quickly after cell exposure: In addition, the CM-H2DCF-DA method

Ineffective in serum, the process of washing cells to remove serum may cause cell loss, resulting in false negatives: Some nanoparticles may be associated with

The DCFH interaction results in partial quenching of fluorescence: Therefore, if the above conditions exist, the negative results of ROS measured by the experiment are not absolute:

of: ISO /T S18827 detects ROS in cells by electron spin resonance (ESR) spectroscopy: This method can be used without interference

Divide into different ROS:

In turn, some factors may cause false positives [10]: Some nanoparticles and dead cells fluoresce: Some nanoparticles can catalyze

CM-H2DCF-DA oxidation: Detection reagents may be preferentially adsorbed on the particle surface [10]: To ensure that positive results are reliable, the test conditions should be

The control group was used to characterize the nanoparticles alone, and to distinguish the fluorescence produced by dead cells from the fluorescence produced by ROS in living cells:

In addition, CM-H2DCF-DA can undergo light-induced auto-oxidation, so its stock solution should be kept in a nitrogen/argon-filled airtight solution regardless of its concentration:

Store in a closed container away from light to prevent light and air from entering:

Therefore, the CM-H2DCF-DA method may only be applicable to specific cell lines and

nanoparticles: Before drawing a final conclusion, it should also be

Additional assays provide further confirmation of the results (see Appendix A for Alternative Cell Lines): When the detection system has the potential to cause false

This is especially important when considering factors for negative or false-positive results: For example, for a positive result, other tests should be found to ensure that the positive result

If not caused by interference: Other factors to consider include: a control group should be used to determine the baseline fluorescence of unexposed cells; cells should be determined

Is it affected by non-toxic nanoparticles; it should be ensured that the ROS positive control reagent and the ROS generated by the nanoparticles are in the assay conditions used

can be detected below: In addition, it remains to be determined whether the nanoparticles interfere with the fluorescence assay, which further contributes to the particle-induced cellular ROS production:

The evaluation result is invalid: If this interference is possible, different doses of sodium can be added to cells exposed to 3-morpholinodiimide (Sin-1):

Control experiments were performed on rice particles to determine whether the nanoparticles quenched fluorescence:

NOTE: This method is considered to be a rapid screening method for nanoparticle-cell interactions: Although screening-like methods are used to assess the cellular effects of nanoparticles

This is critical, but it is also important that the interpretation of the results can be validated by other ROS and related cellular assays:

Nanotechnology Fluorescein Diacetate Detection

Nanoparticle-induced production of reactive oxygen species in macrophages

1 Scope

This document describes how 5-(6)-chloromethyl-2',7'dichlorodihydro-fluorescein diacetate (CM-H2DCF-DA) was detected and evaluated

Methods for the production of reactive oxygen species (ROS) after exposure of RAW264:7 macrophages to nanoobjects, nanoparticles and their aggregates and aggregates:

This document applies to assays using 24-well plates: If other well plates are used, adjust the sample volume and verify the effectiveness of the procedure to

Ensure that test results are credible: