ISO/TS 23034:2021 (E)

Nanotechnologies — Method to estimate cellular uptake of carbon nanomaterials using optical absorption

Contents

Foreword

Introduction

1 Scope

2 Normative references

3 Terms, definitions and abbreviated terms

3.1 Terms and definitions
3.2 Abbreviated terms

4 Method overview

4.1 General
4.2 Optical absorption of carbon nanomaterials
4.3 Optical absorption of biomolecules
4.4 Determination of the concentration of CNMs in dispersion by absorbance
4.5 Case studies

5 Materials and apparatus

5.1 Materials
5.1.1 Chemicals
5.1.1.1 Water, deionized and sterilized pure water, grade 1, in accordance with ISO 3696:1987.
5.1.1.2 Culture medium, with or without serum that meets the growth requirements of the selected cell line.
5.1.1.3 PBS (pH = 7,4).
5.1.1.4 Cell lysis reagent, a colourless buffer solution that contains detergents for mammalian cell lysis/extraction.
5.1.1.5 SDBS solution, SDBS powder dissolved in deionized pure water with concentration of 50 mg/ml.
5.1.1.6 Trypsin-EDTA (0,25 %).
5.1.2 Cell line
5.2 Apparatus
5.2.1 UV-Vis-NIR spectrometer
5.2.2 Cuvette for optical absorption measurement
5.2.3 Incubator, 37 °C, humidified, 5 % CO2/air.
5.2.4 Culture dishes, single-well or multi-well plates can be used. 6 multi-well plates with flat bottom are recommended.
5.2.5 Centrifuge.
5.2.6 Homogenizer.
5.2.7 Cell counter.

6 Cell-uptake testing protocol

6.1 General
6.2 Sample preparation
6.3 Preparation of calibration curve of CNM dispersions
6.4 Cell-seeding
6.5 Treatment of cells with testing suspension
6.6 Cell counting
6.7 Washing cells and preparation of the cell lysate
6.7.1 General
6.7.2 For adherent cells
6.7.3 For floating cells

6.8 Absorbance measurement of the cell lysate

7 Sources of variability

8 Data output

8.1 General
8.2 Data analysis and reporting
8.3 Data sheet format

Annex A (informative) Case study with SWCNTs

A.1 Materials
A.2 Procedure
A.2.1 Preparation of the calibration line
A.2.2 Testing procedure
A.2.2.1 Control test for checking the applicability of the measurement method
A.2.2.2 Cell uptake test
A.3 Test result
A.3.1 Control testing results
A.3.2 SWCNT uptake test results
A.3.2.1 Cell counting
A.3.2.2 Preparation of the cell lysate and absorbance measurement
A.4 Observation of cellular uptake of SWCNTs by Raw 264.7

Annex B (informative) Case study of CNHs

B.1 Materials and equipment
B.1.1 Materials
B.1.2 Cell line
B.1.3 Equipment
B.2 Calibration line of CNHs in aqueous suspension
B.3 Cell uptake test
B.3.1 Cell culture
B.3.2 Cell culture with CNHs dispersion
B.3.3 Cell counting
B.3.4 Preparation of the cell lysates
B.3.5 Absorbance measurement of the cell lysates
B.3.6 Test result

Annex C (informative) Case study of MWCNTs

C.1 Materials and equipment
C.1.1 Materials
C.1.2 Cell line
C.1.3 Equipment
C.2 Calibration line of MWCNTs in aqueous suspension
C.3 Cell uptake test
C.3.1 Cell culture
C.3.2 Cell culture with CNTs test dispersion
C.3.3 Cell counting
C.3.4 Preparation of the cell lysates
C.3.5 Absorbance measurement of the cell lysates
C.3.6 Test result

Page count: 25