

# 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 % CO <sub>2</sub> /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