



International

This is a preview of ISO 4962:2024. [Click here to purchase the full version from the ANSI store.](#)

ISO 4962

Nanotechnologies — In vitro acute nanoparticle phototoxicity assay

*Nanotechnologies — Essai in vitro de phototoxicité aiguë des
nanoparticules*

**First edition
2024-11**



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

This is a preview of ISO 4962:2024. [Click here to purchase the full version from the ANSI store.](#)

Foreword	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms, definitions, symbols and abbreviated terms	1
3.1 Terms and definitions.....	1
3.2 Abbreviated terms.....	2
4 Test method	2
5 Materials and equipment	3
5.1 Materials.....	3
5.1.1 Reagents.....	3
5.1.2 Cell line.....	3
5.1.3 Controls.....	4
5.2 Apparatus.....	5
5.2.1 Laminar flow cabinet, standard biological hazard.....	5
5.2.2 Incubator (37 °C, 95 % humidified, 5 % CO ₂ /air).....	5
5.2.3 Inverted phase contrast microscope.....	5
5.2.4 Centrifuge.....	5
5.2.5 Water bath.....	5
5.2.6 Multiple well plate reader.....	5
5.2.7 Tissue culture flasks.....	5
5.2.8 24 multi-well plates with flat bottom.....	5
5.2.9 Flat bottom 96-well black polystyrene microplate.....	5
5.2.10 8-channel pipette, 20-200 µl Hemocytometer.....	5
5.2.11 Conical tube.....	5
5.2.12 Reservoir.....	5
5.2.13 Vortex mixer.....	5
5.2.14 Refrigerator.....	5
5.2.15 Freezer.....	5
5.2.16 UV crosslinker (capable of light emission at $\lambda=365$ nm), as a UVA source. Irradiation of the test plate should be between 4 and 6 mW/cm ²	5
5.2.17 UV-VIS spectrophotometer, capable of measurements in the wavelength range from 300 to 800 nm.....	5
5.2.18 UV power meter, capable of measurements in the wavelength range from 315 to 400 nm.....	5
6 Sample preparation	5
7 Preparations	6
7.1 General.....	6
7.2 Culture medium.....	6
7.3 Preparation of cell stock culture.....	6
7.4 Verify viable cell growth.....	6
7.5 Irradiation conditions.....	8
7.5.1 UVA source.....	8
7.5.2 Light dose (insolation) measurement.....	8
7.6 Multiple well plate reader.....	8
8 Measurement procedure	8
8.1 Cell seeding (Day 1).....	10
8.2 Incubation of cells with the positive control and NP suspension (Day 2).....	10
8.3 UVA exposure (Day 3).....	11
8.4 Cell viability assay (Day 4).....	11
8.5 Evaluation of artefacts due to possible NP interferences with the MTS assay (in the dark).....	12

This is a preview of ISO 4962:2024. [Click here to purchase the full version from the ANSI store.](#)

9	Report	14
9.1	Test report.....	14
9.2	Report data format.....	15
10	Precision	15
10.1	Repeatability.....	15
10.2	Reproducibility.....	15
Annex A (informative) Schematic diagram of 96-well plate position		16
Annex B (informative) Verification of plate reader uniformity		17
Annex C (informative) Dispersing procedure for TiO₂ nanoparticles in DMEM		18
Annex D (informative) Results of the inter-laboratory test		19
Bibliography		22

This is a preview of ISO 4962:2024. [Click here to purchase the full version from the ANSI store.](#)

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

This is a preview of ISO 4962:2024. [Click here to purchase the full version from the ANSI store.](#)

Phototoxicity (PT) is defined as a toxic response to an organism exposed to a substance, where the response is either elicited or increased (apparent at lower dose levels) after subsequent exposure to light, or that is induced by skin irradiation after systemic administration of a substance. The increasing use of nanomaterials in various industries also leads to increased exposure, especially to skin. Furthermore, some nanomaterials are used in commercial sunscreens. Hence, possible impacts on human health including detrimental chemical reactions in the presence of light (both natural and artificial) or photo-protective effects, is of interest.

PT is based on a quantum phenomenon. The absorption of a photon with sufficient energy generates an electron-hole pair that can migrate to the nanoparticle (NP) surface and react with water and oxygen, thus forming extremely reactive radicals and reactive oxygen species (ROS). Generation of the ROS by some wide-bandgap materials, such as TiO_2 , ZnO , WO_3 , CeO_2 , carbon nanotubes, quantum dots and some metal NPs when illuminated by UV-VIS light, can cause oxidative stress, resulting in toxic effects in living organisms. Absorption of a photon with sufficient energy is the necessary condition for photochemical reactions to induce phototoxic response. Material PT is closely related to photocatalytic activity (PCA). Measurement of PCA under physiological conditions allows for an assessment of its phototoxicity potency (see ISO 20814).

A wide variety of light sources, light exposure levels, cell lines, incubation times, viability assays, used for nanomaterial PT measurement hamper data comparison. Existing PT standard test methods for soluble chemical substances (e.g. OECD 432) are not directly applicable to determine nanomaterial PT. It states that DMSO or EtOH should be used as cosolvents in case the material is not soluble in water with additional tests for cosolvent toxicity. The method is not applicable for particulate materials.

The in vitro NP PT test is intended to evaluate the nanomaterial acute phototoxicity when exposed to a near ultraviolet (UVA) light. Cell viability is assessed at a fixed NP concentration after exposure to six doses of the UVA light.