

ISO 4962:2024-11 (E)

Nanotechnologies - In vitro acute nanoparticle phototoxicity assay

| Contents | | Page |
|--------------------|--|-------------|
| 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 l 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 =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 |
| 8.6 | Data analysis | 13 |
| 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 |