

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