

ISO/TS 19006:2016-08 (E)

Nanotechnologies - 5-(and 6)-Chloromethyl-2,7 Dichloro-dihydrofluorescein diacetate (CM-H2DCF-DA) assay for evaluating nanoparticle-induced intracellular reactive oxygen species (ROS) production in RAW 264.7 macrophage cell line

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Symbols and abbreviated terms	3
5 Materials	3
6 Technical equipment	5
7 Nanoparticle sample preparation	5
8 Preparations	6
8.1 General	6
8.2 Flow cytometry calibration	7
8.3 Experimental culture medium	7
8.4 Reagent preparation	7
8.5 Preparation of cell stock culture	7
8.6 Preparing culture for experiments	7
8.7 Verification of healthy cell growth	8
8.8 Evaluation of nanoparticle interference	9
8.9 Control preparation	9
8.9.1 General	9
8.9.2 Control description	9
8.9.3 Sin-1 stock solution preparation (1 mM)	10
9 Evaluation of nanoparticle impact on ROS generation in cells	10
9.1 Prepare cells in the 24 well plates	10
9.2 Dose the cells with nanoparticles and controls	10
9.3 Expose the cells to CM-H2DCF-DA Assay	11
9.4 Incubate the cells with CM-H2DCF-DA	12
9.5 Flow cytometry analysis	12
10 Data analysis and results	12
Annex A (informative) Alternate cell lines	14
Annex B (informative) Alternate fluorescence characterization techniques	15
Annex C (informative) Suspension preparation and characterization	16
Annex D (informative) Example experimental data from RAW 264.7	17
Bibliography	20