IUF
LEIBNIZ RESEARCH INSTITUTE FOR ENVIRONMENTAL MEDICINE

Cellular DCF-DA assay

Date
07/01/2015

Version
1.0

Drafted within the project "Oxidant generating capacity as a metric to allow grouping of nanomaterials and prediction of human health effects" (nanOxiMet).

This project was supported by the French National Research Agency (ANR) and the German Federal Ministry of Education and Research (BMBF), under the frame of SIINN, the ERA-NET for a Safe Implementation of Innovative Nanoscience and Nanotechnology.

nanOxiMet

Title:	**Cellular DCF-DA assay**
Number:	WP4-1
Date:	07.01.2015
Version:	1.0
Responsible for the implementation of this SOP:	Andrea Neumeyer-Sickinger
	Date / Signature
Author:	*Andrea Neumeyer-Sickinger,* **IUF**
	Name, Organisation
Reviewed:	
	Name, Organisation
Approved:	
	Name, Organisation

Contents

1. Scope

Detection of oxidative stress

2. Basics*

A fluorometric microplate assay for the detection of oxidative stress by detecting oxidation of 2',7'-dichlorofluorescin-diacetate (DCF-DA) into the highly fluorescent compound 2',7' dichlorofluorescein (DCF) due to the presence of reactive oxygen species (ROS) (Figure 1).

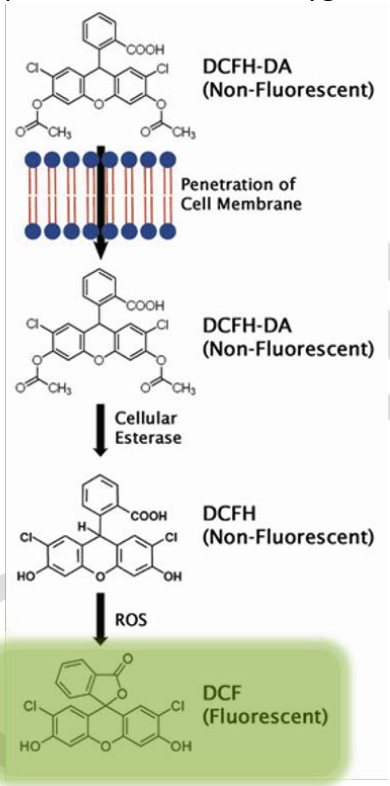


Figure 1. Principle of the DCF-DA assay

3. Materials and instruments

3.1. Materials

• Sterile 96-well microplate with flat bottom	Falcon	#353072
• HBSS	Gibco	#14025100
• 2',7'-dichlorofluorescin-diacetate (DCF-DA)	Sigma	#D6883
• H_2O_2 (30%)	Roth	#8070.2
• DMSO	Sigma	#D5879

3.2. Instruments

Fluorescence reader with a 480 nm excitation filter and a 535 nm emission filter (Tecan Infinite 200) → Hennekamp Building

4. Experimental procedure

4.1. Cell culture

Cells are seeded in adequate amounts (see table 1) in a 96-well plate and cultured (37°C, 5% CO_2, 90% humidity) for x days (see table 1).

Table 1: Cell culture conditions for different cell lines.

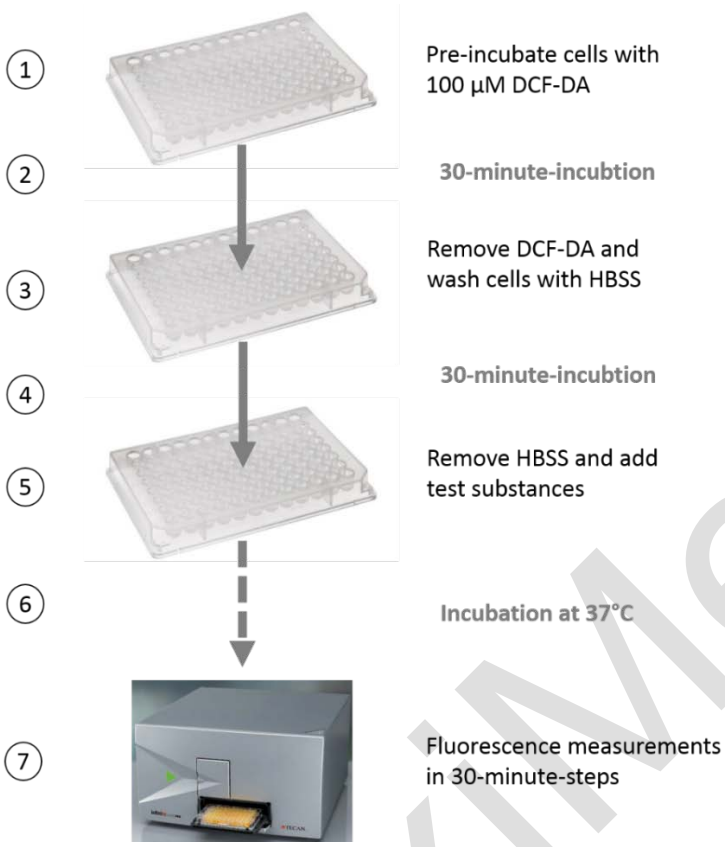
Cell line	Medium	Cells/well	Incubation time
NR8383	F/12K	40.000	48 hours
A549	RPMI	10.000	48 hours

4.2. Preparation of DCF-DA stock solution

- Dilute 50 mg DCF-DA in 2.565 mL DMSO → **40 mM**
- Store solution in aliquots at -20°C
- Solution is stable for about 3 months

4.3. Assay procedure

a. Wash cells with pre-warmed HBSS buffer (NR8383 cells should be centrifuged before cell washing step)
b. Add pre-warmed DCF-DA solution (100 µM in HBSS) to the cells and incubate it for 30 minutes at 37°C, 5% CO_2 and 90% humidity
c. Remove DCF-DA solution and wash cells with HBSS
d. Incubate plate for 30 minutes at 37°C, 5% CO_2 and 90% humidity
e. Remove HBSS and add the test substances (e.g. nanoparticles) diluted in HBSS
f. As positive control add 1 mM H_2O_2 diluted in HBSS
g. Incubate the plate at 37°C, 5% CO_2 and 90% humidity or incubate it in the Tecan Reader for kinetic measurements
h. Measurements should be performed in 30-minute-steps
i. Conversion of DCF-DA into DCF is measured at an extinction of 485 nm and an emission of 530 nm



5. Safety precautions*

Follow the safety information and regulations of the working laboratory and of materials providers. Biosafety level 1 precautions should be followed when handling cells.

6. Waste disposal

Follow the disposal advice from materials providers, if available. Any material containing cells should be discarded as bio hazardous waste.

7. Reference

Foucaud, L., et al. (2007) Measurement of reactive species production by nanoparticles prepared in biologically relevant media. *Toxicol Lett*.

Wang, H. and Joseph, J.A. (1999) Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic Biol Med*. **27,** 612-6.