

Suspending and diluting Nanomaterials

Carbon based nanomaterials

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Document Type	Document ID	Version	Status	Page
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Table of Content

1	Introduction.....	3
2	Principle of the Method	3
3	Applicability and Limitations	3
4	Related Documents	3
5	Equipment and Reagents	4
5.1	Nanomaterial type.....	4
5.2	Equipment	4
5.3	Reagents	4
6	Procedure	4
6.1	Preparation of solvent.....	4
6.2	Preparation of NM stock suspension	4
6.3	Dilution of NM	5
6.3.1	General remarks	5
6.3.2	Example	5
7	Quality Control, Quality Assurance, Acceptance Criteria.....	6
8	Health and Safety Warnings, Cautions and Waste Treatment.....	6
9	Abbreviations	6
10	References.....	7

Document Type	Document ID	Version	Status	Page
SOP	M_NM suspension_carbon based	1.0		2/7

1 Introduction

Cytotoxicity assays are performed with cells growing in cell culture medium. Therefore nanomaterials (NM) have to be suspended in an aqueous medium that allows their application to cells (Schulze et al., 2008). NMs in general and carbon based NMs in particular tend to form agglomerates when put in suspension. Agglomeration behavior depends largely on NM physico-chemical properties and the surrounding liquid. The goal is to find a biocompatible solvent and combine this with ultrasonication to reduce NM agglomeration to a minimum.

2 Principle of the Method

Pluronic F-127 is used as the solvent of choice to suspend carbon based NMs. A 500 µg/ml stock suspension of carbon based NMs is prepared in 160 ppm Pluronic F-127 by 10 min ultrasonication in an ultrasound bath.

3 Applicability and Limitations

Carbon based NMs show a strong tendency to agglomerate. Even after 10 min of ultrasonication suspensions are not stable over time. Larger agglomerates settle down already after a few hours. Therefore suspensions have to be prepared and diluted right before application to cells. Storage of suspensions is not recommended. Furthermore stock concentrations greater than 500 µg/ml are also not advisable. A special dilution procedure has to be followed to assure homogenous distribution of NMs (adapted from Zook et al., 2011).

4 Related Documents

Table 1: Documents needed to proceed according to this SOP and additional NM-related interference control protocols.

Document ID	Document Title

Document Type	Document ID	Version	Status	Page
SOP	M_NM suspension_carbon based	1.0		3/7

5 Equipment and Reagents

5.1 Nanomaterial type

Carbon based NMs. E.g. Carbon nanotubes (CNTs; multi- as well as single-walled), graphitic shells and non-magnetic encapsulates (Bachmatiuk et al., 2013) or carbon black (CB) to name only some.

5.2 Equipment

- Glass tubes
- Ultrasound bath
- Vortex®
- Pipettes
- Micro scales

5.3 Reagents

- Pluronic F-127 [CAS number: 9003-11-6]
- Double-distilled water (ddH₂O)

6 Procedure

6.1 Preparation of solvent

Prepare a 160 ppm Pluronic F-127 solution:

- 160 ppm in ddH₂O: 160 µg/ml (=16 mg/100 ml)

Assure complete dissolution of Pluronic F-127.

Note: Incubation at 37°C for several hours with occasional mixing may be necessary.

Solution can be stored up to 4 weeks at 4°C.

6.2 Preparation of NM stock suspension

Note: Due to agglomeration of carbon based NMs stock concentrations greater than 500 µg/ml are not recommended to be prepared.

- Weigh 2-4 mg of carbon based NM into a sterile glass tube.
- Add appropriate volume of 160 ppm Pluronic F-127 solution to reach a final concentration of **500 µg/ml** NM. Avoid raising dust from NM powder.
- Sonicate for 10 min in an ultrasound bath.
- Use directly for further dilution and application. Do not store.

Document Type	Document ID	Version	Status	Page
SOP	M_NM suspension_carbon based	1.0		4/7

6.3 Dilution of NM

6.3.1 General remarks

Important: "Mixing" in the context of diluting NMs means, the solvent containing tube is put on a continuously shaking Vortex® and the previous sub-dilution (or stock suspension, respectively) is put drop wise into the shaking solvent. The resulting suspension stays on the Vortex® for additional 3 seconds before proceeding with the next sub-dilution (Procedure according to Zook et al., 2011).

The solvent concentration has to be exactly the same in each cellular sample and in the solvent control. Therefore NMs are serially pre-diluted in solvent (160 ppm Pluronic F-127) before final dilution in complete cell culture medium (or any other adequate buffer used in the respective assay). The exact procedure is illustrated in the example (6.3.2) below.

6.3.2 Example

The following six different concentrations of a carbon based NM and the solvent control (7.) shall be finally tested on cells:

1. 80 µg/ml
2. 40 µg/ml
3. 20 µg/ml
4. 10 µg/ml
5. 5 µg/ml
6. 2.5 µg/ml
7. Solvent control: Pluronic F-127

A) In the first step the following sub-dilutions from the 500 µg/ml stock suspension (6.2) are prepared in 160 ppm Pluronic F-127:

2. 300 µl of 500 µg/ml stock suspension (1) are mixed with 300 µl of Pluronic F-127 → 250 µg/ml [2]
3. 300 µl of 250 µg/ml [2] are mixed with 300 µl Pluronic F-127 → 125 µg/ml [3]
4. 300 µl of 125 µg/ml [3] are mixed with 300 µl Pluronic F-127 → 62.5 µg/ml [4]
5. 300 µl of 62.5 µg/ml [4] are mixed with 300 µl Pluronic F-127 → 31.25 µg/ml [5]
6. 300 µl of 31.25 µg/ml [5] are mixed with 300 µl Pluronic F-127 → 15.63 µg/ml [6]

B) The same volume from each of these sub-dilutions [2-6], from the stock suspension [1] as well as from the solvent [7] is then used to prepare the final concentrations in complete cell culture medium or any adequate buffer that is used in the respective assay. See Figure 1.

Document Type	Document ID	Version	Status	Page
SOP	M_NM suspension_carbon based	1.0		5/7

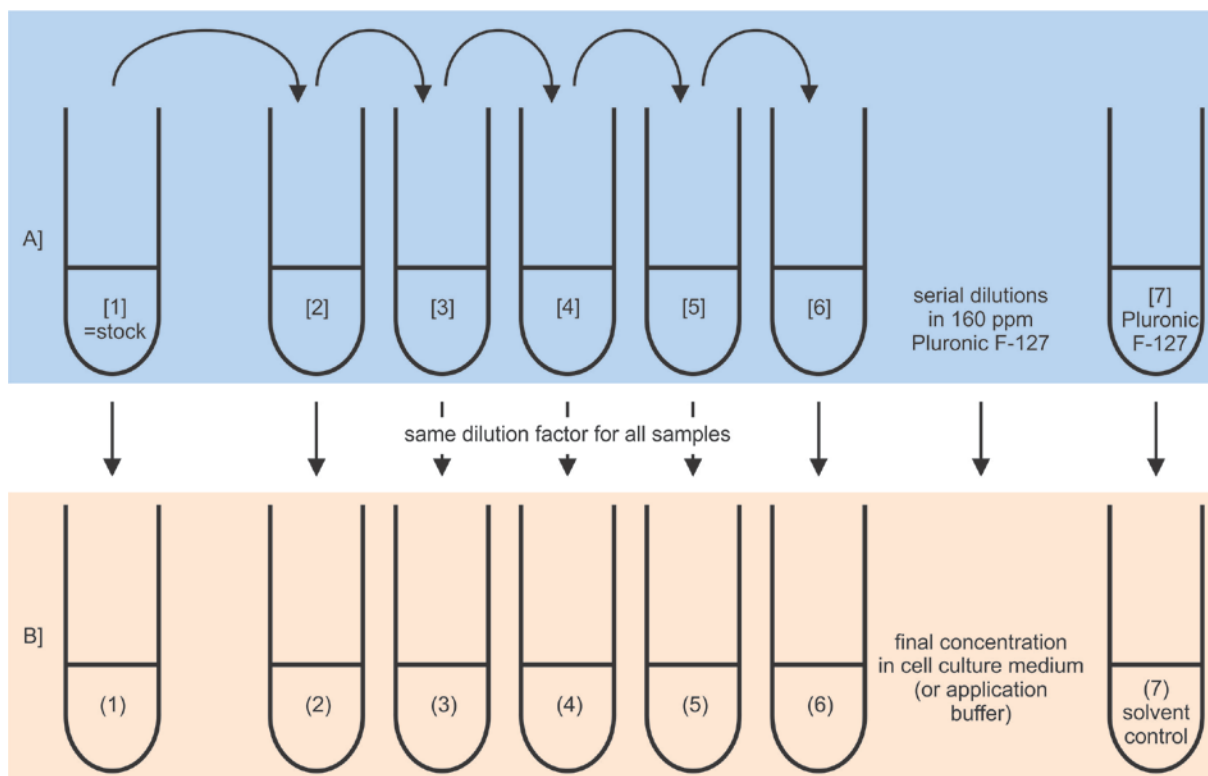


Figure 1: Pipetting scheme for NMs. First serial dilutions in 160 ppm Pluronic F-127 are prepared from the stock suspension [1]. Each of these as well as the stock suspension and the solvent is then further diluted in complete cell culture medium (or any other kind of application buffer). This procedure assures the same amount of solvent in each sample.

7 Quality Control, Quality Assurance, Acceptance Criteria

8 Health and Safety Warnings, Cautions and Waste Treatment

Raising dust from NM powders has to be avoided. Operators should wear masks, gloves and laboratory coat for all handling steps.

Discard NMs separately and according to the institution's rules.

9 Abbreviations

CB	carbon black
CNT	carbon nanotubes
ddH ₂ O	double-distilled water
NM	nanomaterial
ppm	part per million

Document Type	Document ID	Version	Status	Page
SOP	M_NM suspension_carbon based	1.0		6/7

10 References

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Document Type	Document ID	Version	Status	Page
SOP	M_NM suspension_carbon based	1.0		7/7