A common European approach to the regulatory testing of nanomaterials

Standard Operating Procedure

Title	***Micronucleus Assay***
Subtitle	SOP Micronucleus Assay - Lab-Tek® Chamber Slide™ system
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2		
3		
4		

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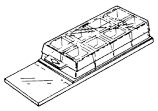
SOP Micronucleus Assay - Lab-Tek® Chamber Slide™ system



1. Cultivation of BEAS-2B cells in Lab-Tek® Chamber Slide™ system

Solutions: BEBM ™ (2-8°C), fibronectin (2-8°C), collagen (2-8°C), bovine serum albumin BSA (2-8°C)

Coating media	Storage	Stock concentration	Solution concentration	V = 45 ml
BEBM ™	2-8 °C			44,2785 ml
Fibronectin	2-8 °C	1 mg/ml	0,01 mg/ml	450 ul
Collagen	2-8 °C	5 mg/ml	0,03 mg/ml	270 ul
BSA	2-8 °C	300 mg/ml	0,01 mg/ml	1,5 ul

For chamber coating, add 200 µl of coating media in each well and incubate for 24 hours in an incubator. Before seeding the cells, suck out all coating media. Seed the cells 24 hours before exposure in 200 µl of cultivation media. Do not exceed 70% confluence.

2. Exposure

Solutions: BEGM™ (37°C), tested compounds

Suck out media from each chamber and add 200 µl of fresh pre-warmed media with tested compounds. For 28 hour exposure add cytochalasin-B (final concentration 1 µg/ml). For 48 hour exposure add cytochalasin-B (final concentration 1 µg/ml) after 20 hours.

3. Hypotonic solution and fixation solution

Solutions: Hypotonic solution (37°C), fixation solution (2-8°C), methanol (2-8°C), distilled H_2O

Fixation solution has to be prepared fresh, pre-cooled to 2-8°C. Hypotonic solution can be stored up to 6 months. Suck out media from each chamber and add 200 µl of pre-warmed hypotonic solution for 3,5 minutes. Suck out a hypotonic solution and add 200 µl of fixation solution (2-8°C) for 1-2 minutes. Suck out fixation solution and add 200µl of methanol (2-8°C) for 1-2 minutes. Suck out all liquids from the chamber and let it dry at least for 20 minutes before staining.

Fixation solution (2-8°C)				
Methanol	3 ml	9 ml	15 ml	24 ml
CH_3COOH	1 ml	3 ml	5 ml	8 ml
Volume	4 ml	12 ml	20 ml	32 ml

Hypotonic solution (37°C)			
KCl	0,55 g	2,75 g	5,5 g
Distilled H_2O	100 ml	500 ml	1000 ml
Volume	100 ml	500 ml	1000 ml

4. Staining (5% Giemsa solution)

Solutions: distilled H_2O, Sörensen buffer, Giemsa

Sörensen buffer (2-8°C)		
Solution A	KH_2PO_4 (g)	1,376
	distilled H_2O	400 ml
Solution B	Na_2HPO_4 . 12 H_2O (g)	5,52 ml
	distilled H_2O	600
Volume		1000 ml

5% Giemsa solution	
distilled H_2O	80 ml
Sörensen buffer	15 ml
Giemsa	5 ml
Volume	100

Mix Solution A and B and store Sörensen buffer of to 6 months in the refrigerator. For staining, immerse fixated samples to 5% Giemsa solution for 3,5 minutes and then was briefly with distilled water.

5. Scoring

Visual scoring using the microscope is used to analyse the binucleated cells in final magnification 1000x. A total of 3 x 500 binucleated cells per each tested compound is evaluated and the cytokinesis-block proliferation index (CBPI) is calculated to control for cell division. The aberrant cells are recorded using a camera. The results are expressed as a percentage of BNC with MN (% ABB).