Standard Operating Procedure

SOP details

Title	Sterilization of TopoChip
Description	This SOP describes how to sterilize the TopoChip using 70 % ethanol.
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SOP number	2.1
Version number	1

	Name	Date	Signature
Prepared	Phani Krishna Sudarsanam	07-05-2020	
Reviewed	Jan de Boer	18-03- 2021	
Reviewed			
Authorized	Jan de Boer	20-4-2022	

Version changes

Version	Name	Date	Changes made
1	Phani Krishna Sudarsanam	07-05-2020	Made in TU/e
2	Jan de Boer	16-4-21	With track changes
3			
4			
5			

Table of contents

1 Purpose

This SOP describes how to sterilize the TopoChips before using them for cell culture experiments.

2 Principle

TopoChips are produced under septic conditions in the microfabrication lab, so they must be sterilized before being used for cell culture experiments. Immersion in 70% ethanol ensures that there is no contamination on the TopoChips.

3 Before You Start

This SOP precedes TopoChip screening. Before using this SOP, one should be familiar on how the TopoChips are fabricated and which kind of polymer is used for making them that are used for the cell culture experiments and whether ethanol sterilization is compatible with the chemistry of the TopoChip. Generally, TopoChips made of Polystyrene, Polylactic acid (PLA), Poly(styrene-block-isobutylene-block-styrene) or SIBS can be used to sterilize using 70 % ethanol.

4 Required materials

4.1 Workplace

This SOP can be performed in the Cell lab (Gemini-Zuid 4.01 & 4.02). Follow the safety protocols instructed by the lab managers and perform the experiment accordingly in allocated locations in the lab.

4.2 Equipment and disposables

- Class II-biological safety cabinet with UV light
- Incubator with 5% CO2 and set to 37 °C
- Conical flask for waste with chlorin tablets
- Biohazard waste bag and standard
- Paper tissues
- Micro pipettes
- Sterile pipette tips (1000 µl)

4.3 Reagents

- 70% ethanol at room temperature for sterilization
- Phosphate Buffered Saline (sterile PBS, Sigma, Cat. No: D8537)
- 6-well plate
- Marker pens

5 Procedure

5.1 Working procedure

- All the steps are performed in a laminar air flow safety cabinet.
- A new sterile 6-well plate is opened and properly labelled using a marker.
- Place TopoChip in a well.
- 500 µl of 70 % ethanol is added to the well and left in the laminar air flow safety cabinet for 30 minutes. Make sure that the TopoChip is submerged in the ethanol solution.
- After 30 minutes of ethanol treatment, ethanol is aspired out of the well, and TopoChips are washed with 1 ml of sterile PBS for three times.
- PBS is aspirated out of the wells and the well plate with TopoChips inside are ready for cell seeding.

5.2 Safety.

Work in the Cell lab in Gemini-Zuid according the safety regulations. Follow the instructions given in the lab introduction by the lab managers

6 Waste

When working in the cell lab, handle waste according to guidelines which are labelled at the waste disposal based on its categories as given below in the table 1

7 References

SOPnr	Title
2.2	Cell seeding for TopoChips

Technische Universiteit Eindhoven University of Technology

Categorisation of hazardous waste substances

HELP PROTECT SAFETY, HEALTH AND THE ENVIRONMENT

					N/A	N/A
Category I (diluted) inorganic acids with heavy metals pH ≤ 7	*Category II* (diluted) inorganic caustic solutions pH ≥ 7	*Category III* low halogen content organic substances	*Category IV* high halogen content organic substances	*Category V* special waste substances	N/A	*Category VI* waste substances containing special risks
laboratory chemicals (original packaging for chemicals still containing residues), contact Waste management & logistics, phone no 4343						Radioactive substances
Diluted inorganic acids (nitric acid, hydrochloric acid, etc.)	Diluted inorganic hydroxides	Liquid organic substances (alcohol, acetone, toluene, etc.)	Liquid halogenated organic substances (i.e. substances containing fluorine, chlorine, bromine or iodine)	Preparations and specimens		Biological waste
Heavy metal cations in solution (e.g. zinc, copper, nickel, lead)	Heavy metal ions in solution (e.g. zinc, copper, nickel, lead)	Solid and pasty low-halogen organic substances (including plastics)	Solid and pasty high-halogen organic substances (including plastics)	Chemically contaminated packaging/equipment		gas cylinders and pressurised containers
Cations and anions of heavy metals	Anions of metalloids	Mineral lubrication and system oils (such as sump oil)	Pesticides (containing halogen-based compounds)	Small, hazardous waste		
Solutions containing fluoride	Solutions containing cyanide	Oil emulsions (such as drill, grinder, roller and cutter oils containing water)	Waste oils contaminated with substances containing halogens	All other chemical waste that cannot be placed in categories I to IV or VI . *See the TU/e Science Park Waste Substances Manual*		For the disposal of the waste substances listed above, contact the SBD, BVF or Waste management & logistics
Waste liquid from plating and pickling baths (acid), fixing salt	Photograph developer and activator	Pesticides (halogen-free)				

For the disposal of the waste substances listed above, contact the Waste management & logistics

General tips:
Read the 'Waste Disposal' instructions and the TU/e Science Park Waste Substances Manual (AMSO website)
Make sure waste is categorised correctly
Correctly label waste
Watch out for accidental reactions (if you have any doubts, see the list of hazardous combinations in the TU/e Science Park Waste Substances Manual)
Be aware of the risks associated with Category III and IV substances
Work neatly and tidily, use personal protective equipment
Use the correct packaging for collecting waste
Always close (empty) packaging
If possible, only open packaging in a fume cupboard
Fill packaging to a maximum of 90% (up to the indicator strip)
For the temporary storage of packaging, follow the 'Storage of hazardous substances in accordance with PGS 15' procedure (Appendix 1 of the TU/e Science Park Waste Substances Manual)
Call the TU/e Waste Centre before collection
If you have any doubts about specific types of waste or questions concerning the above procedures, etc., you should always contact your faculty's local health, safety and environment coordinator

Important telephone numbers:
Waste Management & logistics (AMSO) (040 247) 4343
Radiation Protection Service (SBD) (040 247) 3500 (if there is no answer call 3355)
Biological agents safety officer (BVF) (040 247) 3555 (if there is no answer call 3500)
(040 247) 2239
Emergency telephone number (040 247) 2222

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Table 1: Categories of hazardous liquid waste