Standard Operating Procedure

SOP details

Title	Immunofluorescence staining of TopoChips
Description	This SOP describes how to do an immunofluorescence staining of a TopoChip. It can be adapted to the antibodies that are used for specific screen.
Author	Phani krishna Sudarsanam
SOP number	2.3
Version number	2

	Name	Date	Signature
Prepared	Phani krishna Sudarsanam	30-04-2020	
Reviewed	Sultan Gulce Iz	12-05-2020	
Reviewed			
Authorized	Jan de Boer	20-4-2022	

Version changes

Version	Name	Date	Changes made
1	Joris van Ark	24-10-2017	First version
2	Phani Krishna Sudarsanam	28-04-2020	Modified substantially in TU/e
3	Jan de Boer	14-4-21	Added comments in track changes

Table of contents

1 Purpose

This SOP describes a general protocol for immunofluorescence staining. It can be adapted to antibodies for screen-specific proteins based on antibody/antigen specific conditions.

2 Principle

This is a general immunofluorescence staining protocol with nuclear and cytoplasmic counterstaining using Phalloidin and DAPI. For each staining, the antibody dilution, time and temperature of incubation steps and blocking strategy need to be optimized. The conditions described in this protocol do work for quite a few different antibodies with cells on glass slides or polystyrene and are therefore a good starting point for the optimisation of your own protocol.

3 Before you start

This SOP is meant to be used as a framework to be optimized for each antibody. Before the experiment, one is required know how to work in the cell culture lab and handle basic cell culture experiments. Knowledge should be obtained on cell fixation strategies, how to find the right antibody dilution and strategies for antigen retrieval and blocking reagents. Selection of the right primary and secondary antibodies is also important to have right results for the bioassay setup. Information on these topics can be found at:

1. https://www.abcam.com/protocols/antibody-dilutions-and-titer
2. http://docs.abcam.com/pdf/protocols/immunocytchemistry-icc-protocol.pdf
3. https://www.abcam.com/protocols/choosing-an-antibody

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

Vacuum system
Laminar flow cabinet
Water bath
Stain less steel tweezers
Micro pipettes

4.3 Reagents

Material	Company	Storage (stock)	Order No
Phosphate Buffered Saline (PBS, 1x)	Gibco		#10010
3.7% formaldehyde	Sigma		F8775-500ml

Triton-X100	Sigma		T878-250ml
Bovine Serum Albumin (BSA)	Sigma		A9647-100g
Phalloidin TRITC	Invitrogen		A12379
DAPI	Sigma-Aldrich		32670-5MG-F
Primary antibody	---		---
Secondary antibody	---		---
Mounting medium: Mowiol	Sigma		
Microscopy slides	Thermo Scientific		10019419
Cover slips (24x50 mm)			

Information about safety and risk of chemicals can be found in the cell lab (Registration of hazardous chemicals)

4.4 Preparation of working solutions

3.7% Formaldehyde/PBS: Work in the fume hood. Dilute the stock 10x in PBS.

0.1% Triton X-100: Triton X-100 is very viscous, therefore heat it to 50°C for 10 minutes on a hot plate before pipetting. Cut off a small piece of a blue pipette tip in the fume hood and pipette the stock. Pipette 100 µL in 100 ml of PBS in a glass flask and stir it to mix it well.

Blocking buffer: 3% BSA in PBS. Don't make more than necessary since BSA is quite expensive. If you make 20mL than weigh 0.4g of BSA and dissolve this in 20mL of PBS. Can be stored in aliquots at -20°C.

5 Procedure

5.1 Working procedure

This working procedure is written for a 6-well plate to stain TopoChips

1. Remove culture media by aspiration using the sterile pasture pipette in the cell laminar flow hood.
2. Wash 3 times with 1 mL of PBS at room temperature.
3. Fixation: add 1 mL of 3.7% formaldehyde/PBS to each well from the corner of each well plate while avoiding the TopoChip in the well and incubate for 15 minutes at room temperature.
4. Aspirate the formaldehyde and wash 3 times with 500 µL of PBS. Now the well plate can be moved to the working bench in the lab of room 4.02 in cell lab where staining can be performed.
5. Add 500 µL of 0.1% TX100 to each sample, incubate for 10 min at room temperature.
6. Aspirate and wash 3 times with 500 µL of PBS.
7. Add 500 µL of blocking buffer to each sample, incubate for one hour at room temperature. Make sure to thaw the blocking buffer to room temperature.
8. The blocking buffer is aspirated out from the wells and washed three times with 500 µL of PBS.

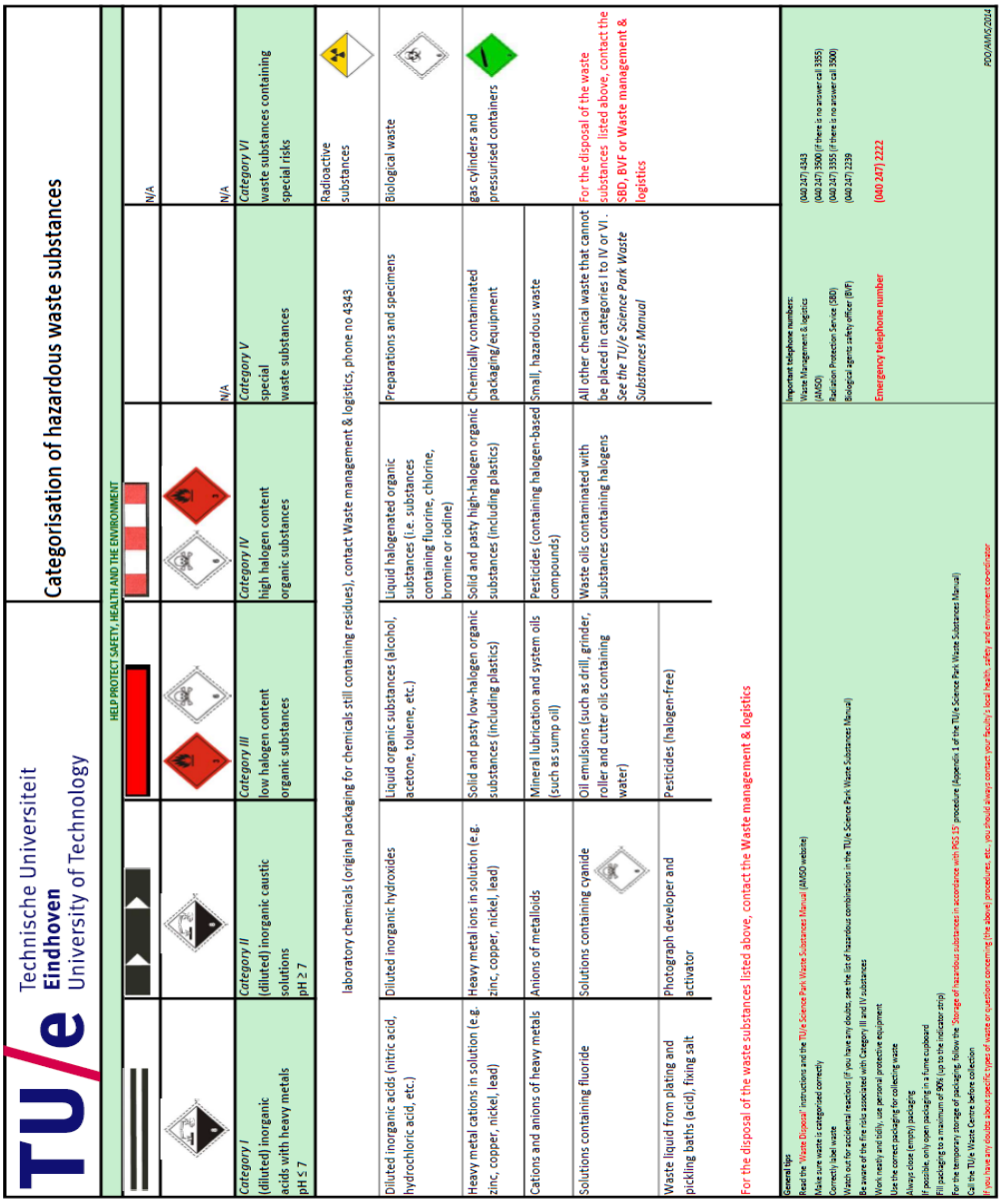
9. Next, incubate with 250 µL of primary antibody (or antibodies) diluted in blocking buffer, incubate for 1h at room temperature or at 4°C overnight .
10. Wash 3x with 500 µL of PBS.
11. Incubate with 250 µL of secondary antibody diluted in blocking buffer, incubate for 1h at room temperature in dark.
12. Wash 3x with 500 µL of PBS at room temperature.
13. Incubate with Phalloidin-TRITC (1: 200) diluted in blocking buffer for 45 minutes at room temperature.
14. Wash the cells 3x with 500 µL of PBS at room temperature.
15. Incubate cells for nuclear staining using DAPI with 100 ng/ml diluted in blocking buffer for 15 minutes at room temperature.
16. Wash 3x with 500 µL of PBS at room temperature.
17. Pipette a drop of mounting medium on a clear glass slide, take the TopoChip between tweezers put istained side of the TopoChip facing the mounting medium onto the glass slide slowly by avoiding the air bubbles. Be careful to not scratch the TopoChip while using the tweezers touching the surface topographies
18. Make sure that the orientation fo the TopoChip is always maintained exactly same for all TopoChips while mounting.
19. **For convenience, mounting of the TopoChip is done such that the flat unpatterned TopoUnit is always at the top right corner so that while imaging it is always on the bottom right corner.**
20. Now a thin glasscoverslip is placed on top of the TopoChip slowly to avoid air bubbles. Do not press the coverslip on the TopoChip.
21. Sealing the coverslips is done by applying a small drop of nail polish on both sides of the coverslip.
22. The mounted samples are stored overnight in the dark at 4°C to dry before imaging.
23. Fluorescence can be maintained without any signal decay for almost 7-9 months if the mounting medium is sealed properly with the coverslip.

5.2 Safety

Work in the Cell lab in Gemini-Zuid according the safety regulations. Staining dyes such as DAPI are hazardous to your health. Be aware of it and work accordingly to the safety instructions in the datasheets. The table showing the symbols representing the different hazardous substance can be seen below (Table 2). Follow the instructions given 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.



TU/e Technische Universiteit Eindhoven University of Technology					Categorisation of hazardous waste substances				
HELP PROTECT SAFETY, HEALTH AND THE ENVIRONMENT								N/A	
						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			*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									

Table 1: Categories of hazardous liquid waste

CLP	Hazard	Advice
	May explode if exposed to fire, heat, shock, friction	Avoid ignition sources (sparks, flames, heat) Keep your distance Wear protective clothing
	Flammable if exposed to ignition sources, sparks, heat. Some substances with this symbol may give off flammable gases in contact with water.	
	Can burn even without air, or can intensify fire in combustible materials.	
	Contains gas under pressure. Gas released may be very cold. Gas container may explode if heated.	Do not heat containers Avoid contact with skin and eyes.
	Corrosive material which may cause skin burns and permanent eye damage. .	Avoid contact with skin and eyes. Do not breathe vapours or sprays. Wear protective clothing.
	May corrode metals	Keep away from metals.
	Toxic material which may cause life threatening effects even in small amounts and with short exposure.	Do not swallow the material, allow it to come into contact with skin or breathe it.
	May cause serious and prolonged health effects on short or long term exposure.	Do not swallow the material, allow it to come into contact with skin or breathe it.
	May cause irritation (redness, rash) or less serious toxicity.	Keep away from skin and eyes.
	May damage the ozone layer	Avoid release to the environment
	Toxic to aquatic organisms and may cause long lasting effects in the environment.	

Table 2: Signs used for different hazardous chemicals