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OHSU SenNet Senescence-Associated Beta-Galactosidase (SA b-Gal) Staining of Carboxymethyl Cellulose (CMC) Embedded Skin Tissue Sections on Polyethylene Naphthalate (PEN) Coated Slides

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Cellular Senescence Net…

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We use this protocol and it's working

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Abstract

Senescence-associated beta-galactosidase staining is a common staining technique to detect senescent cells. This protocol describes SA b-Gal staining of mouse skin tissue sections from CMC embedded blocks onto PEN slides which enable the combined use of laser capture microdissection (LCM) and UV laser cutting for downstream mass spectrometry analysis.

TISSUE SECTIONING AND EMBEDDING PROCEDURE

1 Collecting the Skin

1.1 From the wet lab take petri dishes for each mouse that you are collecting tissue from and place in a bucket of ice

1.2 Once in mouse room, follow protocol for euthanizing the mice

1.3 Once the mice are euthanized, use an electric razor to shave the backs of each mouse

1.4 Apply a generous amount of Nair onto the back of each mouse and let sit for ~ 1 min

1.5 Using a paper towel, carefully wipe the Nair off the mouse's back and spray with ethanol to clean area

1.6 Using scissors and a pair of tweezers, cut a rectangle shape of the dorsal skin (from upper shoulders down to lower hips) and place epithelial side up onto the appropriately labeled petri dish

1.7 Bring the tissue samples back to the wet lab and prepare an ice bucket of dry ice

1.8 Using a clean razor blade, cut off the uneven edges of each tissue segment and then cut into 6-8 smaller rectangle pieces that are all roughly the same size

2 Embedding the Skin in CMC

2.1 Label two Tissue-Tek Cryomolds per each mouse and pipette Carboxymethyl Cellulose (CMC) media into them until they are halfway full

2.2 Using tweezers, carefully pick up the cut sections of tissue and place them vertically in the CMC media, making sure they are all oriented the same direction. Place 3-4 tissue pieces in each cryomold

2.3 Add more CMC media once tissue pieces are in the cryomold.

Note: Try to have the tissue pieces not sink towards the bottom, and have them all relatively on the same plane

2.4 Once tissue sections are vertical, place the cryomolds onto dry ice to harden the CMC media. Add enough CMC media to where you do not see the tissue sections

2.5 Allow the cryomolds to harden on dry ice for at least 15 minutes before placing in -80°C freezer for long term storage

3 Tissue Sectioning on the Cryostat

3.1 Take CMC embedded tissue sections out of -80°C freezer, remove block from the cryomold and mount onto pedestal in the Cryostat chamber using a small amount of OCT media to adhere it. Allow to set for ~15-30min
Note: Cryostat should be set at -20°C

3.2 Cut tissue sections at 10 µm thickness in the cryostat at -20°C and place onto Polyethylene Naphthalate (PEN) slides
Note: Make sure that the tissue is placed onto the membrane bound side of the slid

3.3 Move slides into -80°C freezer until ready for staining procedure

STAINING PROCEDURE

4 Fixation of Slides

4.1 Take tissue slides from -80°C freezer and immediately place in fixation solution for 10 minutes at 4°C on shaker
Fixation Solution
0.2% glutaraldehyde
2% glycerol
in PBS

4.2 Wash slides in 2% glycerol in PBS for 2 minutes on shaker at room temperature

5 X-gal Staining

5.1 Make staining solution

	Actual Con.	Stocks	Amount needed for 5ml
	1mg/ml X-gal	40mg/ml	125µl
	150mM NaCl	5M	150µl
	2mM MgCl2	1M	10µl
	5mM K3Fe(CN)6	100mM	250µl (protect from light)
	5mM K4Fe(CN)6	100mM	250µl (protect from light)
	40mM NaPi pH 6.0	0.1M	2 ml
	H2O		2.217 ml

* K3Fe(CN)6 and K4Fe(CN)6 should be protected from light
* Keep X-gal stock at -20°C
To make up 0.1M NaPi stock (remake every 2 months):

	Actual Con.	Stocks	Amount needed for 10ml
	Na2HPO4	1M	125µl
	NaH2PO4	1M	880µl
	H2O		8.995 ml

5.2 Check the pH of the NaPi stock using a pH meter, it should be at 6.0

5.3 Check the pH of the final staining solution, for best results the pH should be at 6.0

5.4 Filter the staining solution using a .45um filter to prevent crystals from forming. **Protect from light**

5.5 Use a Kimwipe to absorb excess PBS. Do not allow the tissue section to dry out

5.6 Using a PAP pen, draw a square around the tissue and place ~200ml of staining solution onto tissue and incubate for 6-24 hours at 37°C in a humidified chamber/slide holder
Note: The timing depends on the negative (non-senescent) control tissue, you will

need to adjust the timing based on what is appropriate for your sample

5.7 To stop staining reaction, fix again for 10 minutes in fixation solution at room temperature on shaker

5.8 Wash slides in 2% glycerol in PBS for 5 minutes on shaker at room temperature

6 Nuclear counter stain: Nuclear fast red is a counterstain that targets nucleic acids and helps identify the nucleus of cells

6.1 Place slides in Nuclear Fast Red for 10 minutes at room temperature

6.2 Rinse slides with tap water 3 times

6.3 Place slides in tap water for 3 minutes on shaker

6.4 Let slides air-dry at room temperature
Note: For laser ablation, cover slip and mounting media were not used.