REED LABORATORY – CONFOCAL IMMUNOFLUORESCENCE
UIC College of Dentistry

[0] Fix tissue in 4% PFA overnight
Embed in Paraffin and section

[1] **DEPARANNIZING – TIME: 1HR**
Fill each of the containers
Xylenes – 15 min
100% ETOH – 5 min
100% ETOH – 5 min
95% ETOH – 5 min
70% ETOH – 5 min
1x PBS – 15 min

[2] **SODIUM BOROHYDRIDE – TIME: 2 X 30 MIN**
Make fresh, it will start to bubble:
- 1x PBS – 15ml
- NaBH4 – 0.075g (sigma 452882 – 100g)

Place slides in mailer
Change after 30 min

[3] **CITRATE BUFFER – TIME: 10 MIN HEATED, 30 MIN ROOM TEMP**
Preheat Heat tap water to a boil for 6 min in microwave
Place on hotplate and bring to boil
Fill mailer with 0.01 M Citrate Buffer
Place in boiling water, and take off of the heat source
Take out of heated water after 10 min
Allow to cool to room temp for 30 min
- Citrate Buffer, Ph 6 STOCK SOL:
  - 1.05g citric acid, monohydrate (Fisher BP339,500)
  - 500ml Dh2O
  - ADJUST TO Ph 6 WITH 5m NaOH (5M → 20g NaOH in 100 ml Dh2O)

[4] **TRITON WASH – TIME: 3 X 5 MIN**
Wash in the mailer
0.5% (v/v) Triton-x100
- To make this, cut the pipette tip off
- Slightly warm PBS before adding Triton
- Eject pipette tip right into PBS

[5] **MEOH WASH -TIME: 3 MIN**
Fill mailer with -20 MEOH
Place slides into MEOH

[6] **SERUM BLOCKING – TIME: 1 HR**
Fill mailer with blocking solution, transfer slide right from MEOH into blocking solultion
- 5% Serum Blocking Solution
  - 1x PBS – 19 ml
  - Animal Serum – 1 ml

We almost exclusively use DONKEY SERUM (Sigma, pn)
Save 0.5 ml of serum for the primary antibody step

[7] **PRIMARY ANTIBODY – TIME: O/N**
Transfer slides to hydrator
Wipe off excess solution around tissue
Pipette 250 uL of antibody solution onto slide
Cover each slide with a slip of PARAFILM
Cover hydrator, and place in 4 degree refrigerator overnight
Primary Antibody Solution [PER SLIDE]
5% Serum Blocking Solution - 6.25 µl
1x PBS -191.25 µl
0.5% Triton – 50 µl
Antibody1 (1:200 dilution) – 2.5 µl
Antibody2 (1:200 dilution) – 2.5 µl

DAY 2

[8] **TRITON WASH – TIME: 3 X 5MIN**

[9] **SECONDARY ANTIBODY STEP – TIME: 2 HRS**
Turn off the overhead lights
Transfer slides to hydrator
Wipe off excess solution around tissue
Pipette 250 µl of secondary antibody solution onto slide
Cover hydrator, and place in drawer out of light
Secondary Antibody Solution [PER SLIDE]
Animal Serum - 5 µl
1x PBS - 243.75 µl
Secondary Antibody1 (1:200 dilution) – 1.25 µl
Secondary Antibody2 (1:200 dilution) – 1.25 µl

[10] **PBS WASH – TIME: 3 X 5 MIN**

[11] **DAPI STAIN – TIME: 5 MIN**
Transfer slides to hydrator
Wipe off excess solution around tissue
Pipette 250 uL of DAPI solution onto slide
DAPI Solution
1x PBS – 250 µl
DAPI – 0.25 µl

[12] **COVER SLIPPING**
Cover with prolong gold antifade (p36930, Invitrogen)
Pipette 100 µl on to the coverslip
Cut the tip of the pipette

STORE THE SLIDES IN THE 4 DEGREE FRIDGE O/N
SEAL WITH NAIL POLISH THE NEXT DAY