

REED LAB - WHOLE MOUNT IMMUNO PROTOCOL FOR 2-PHONTO MICROSCOPY
UIC College of Dentistry

- 1) 4% PFA
- **TIME overnight**

- 2) Transfer PBS → MeOH 50%, 70%, 95%, 100%
- **TIME 10 min each**

- 3) Dents Bleach
- **TIME 1 day at 37C rocking**
Solution:
MeOH - 400 ul
DMSO - 100 ul
30% H₂O₂ – 100 ul

- 4) 100% MeOH wash
- **TIME 1 hour**

- 5) Equilibrate MeOH → PBS (50%, 70%, 95%, 100%)
- **TIME 10 min each**

- 6) PERMILABILIZE with PBS, 2% Triton, 20% DMSO
- **TIME 1 day**
Solution:
10 ml PBS
20 uL triton
200 ul DMSO

- 7) Blocking in TN solution
- **TIME 4 day at 37C rockin**
TN Solution:
PBS 100 mL
0.1M Tris – 12.114 g
0.15M NaCl – 5.84 g
With HCl adjust to 7.4 pH
Blocking Solution 3%:
Add 3 ml of Donkey Serum

- 8) Incubate Primary Antibody (1:200)
- **TIME 10 DAYS at 37C rocking**
Solution:
500 uL of Blocking in TN solution (above)
5 ul of Collagen VI (because its diluted)

- 9) wash in PBS with 1% Triton
- **TIME 3 x 30 min**

- 10) Incubate Secondary Antibody (1:200) in TN+BSA solution
– **TIME 10 DAYS at 37C Rocking**
- 11) wash in PBS with 1% Triton
– **TIME 3 x 1 hr**
- 12) CLEAR? BABB?