

iDISCO+ Daily Worksheet

* All sections in one 5ml screw-cap tube

** 6-7ml of solutions in large, 5ml screw-cap or eppendorf tubes

*** Carefully decant and immediately add new solution when changing solutions in same tube

**** When transferring to new tube, pour sections into glass dish and use flat tweezers or spatula to move to new tube

Day 1 (Mon _____)

- Dehydration

- 20% MeOH for 60min @ RT, on nutator
- 40% MeOH for 60min @ RT, on nutator
- 60% MeOH for 60min @ RT, on nutator
- 80% MeOH for 60min @ RT, on nutator
- 100% MeOH for 60min @ RT, on nutator

- Transfer to new 5ml tube
- 100% MeOH for 60min @ RT, on nutator

- Transfer to new 5ml tube
- 66% DCM + 33% MeOH O/N @ RT, on nutator
 - 24ml DCM (draw out using pipetting needle and syringe and only use conical tubes)
 - 12ml 100% MeOH
 - Vortex and pipet 6ml per 5ml tube

Day 2 (Tues _____)

- Background Bleaching

- Transfer to new 5ml tube
- 100% MeOH for 60min @ RT, on nutator
- 100% MeOH for 60min @ RT, on nutator
- Place samples on ice for 30min

- 5% H₂O₂ in MeOH O/N @ 4C
 - 6ml 30% H₂O₂
 - 30ml 100% MeOH
 - Invert to mix and pipet 6ml per 5ml tube
 - Make solution on ice and let sit for 15min before transferring sections

Day 3 (Wed _____)

- Rehydration and Permeabilization

- Transfer to new 5ml tube
- 80% MeOH for 60min @ RT, on nutator
- 60% MeOH for 60min @ RT, on nutator
- 40% MeOH for 60min @ RT, on nutator
- 20% MeOH for 60min @ RT, on nutator
- 0.01M PBS for 60min @ RT, on nutator

- Transfer to new 5ml tube
- PBS-T (0.01M PBS + 0.2% Triton X-100) for 60min @ RT, on nutator
- PBS-T (0.01M PBS + 0.2% Triton X-100) for 60min @ RT, on nutator
- Permeabilization Solution O/N @ 37C, on nutator
 - 32ml PBS-T
 - 0.92g Glycine
 - 8ml DMSO
 - 160ul 5% NaAzide solution
 - Invert or place on nutator until in solution and pipet 6ml per 5ml tube

Day 4 (Thur)

- Blocking

- PTwH (0.01M PBS + 0.2% Tween-20 + 10mg/ml Heparin + 0.02% NaAzide) for 10min @ RT, on nutator
- PTwH for 10min @ RT, on nutator

- Transfer to new 5ml tube
- Blocking solution O/N @ 37C, on nutator
 - 30ml PBS-T
 - 3.5ml DMSO
 - 2.1ml Normal Goat Serum
 - 140ul NaAzide
 - Invert to mix and pipet 6ml per 5ml tube

Day 5 (Fri)

- Primary Antibody (Rabbit anti-v5), day 1 of 3

- Primary antibody solution with Rabbit anti-v5 O/N @ 37C, on nutator
 - Make Primary Buffer
 - 92ml PTwH
 - 5ml DMSO
 - 3ml Normal Goat Serum
 - 400ul NaAzide
 - Invert to mix and store in 4C
 - Primary antibody solution
 - 32 ml Primary Buffer
 - 64 ul Rabbit anti-v5 (Bethyl, 1:500)
 - Invert to mix and pipet 6ml per 5ml tube

Day 6 (Sat)

- Primary Antibody (Rabbit anti-v5), day 2 of 3

- Primary antibody solution with Rabbit anti-v5 O/N @ 37C, on nutator
 - 32 ml Primary Buffer
 - 64 ul Rabbit anti-v5 (Bethyl, 1:500)
 - Invert to mix and pipet 6ml per 5ml tube

Day 7 (Sun)

- Primary Antibody (Rabbit anti-v5), day 3 of 3

- Primary antibody solution with Rabbit anti-v5 O/N @ 37C, on nutator
 - 32 ml Primary Buffer
 - 64 ul Rabbit anti-v5 (Bethyl, 1:500)
 - Invert to mix and pipet 6ml per 5ml tube

Day 8 (Mon)

- Washing

- Transfer to new 5ml tube
- PTwH for 10min @ RT, on nutator
- PTwH for 10min @ RT, on nutator
- PTwH for 10min @ RT, on nutator
- PTwH for 60min @ RT, on nutator
- PTwH for 120min @ RT, on nutator
- PTwH for 120min @ RT, on nutator
- PTwH for O/N @ RT, on nutator

Day 9 (Tues)

- Secondary Antibody (Goat Alexa647 Anti-Rabbit) and NeuroTrace Green, day 1 of 3

- Secondary antibody solution with Goat Alexa647 Anti-Rabbit and NeuroTrace Green O/N @ 37C, on nutator
 - Make Secondary Buffer
 - 97ml PTwH
 - 3ml Normal Goat Serum
 - 400ul NaAzide
 - Invert to mix and store in 4C
 - Secondary antibody solution with NeuroTrace
 - 32 ml Secondary Buffer
 - 64 ul Goat Alexa647 Anti-Rabbit (Invitrogen, 1:500)
 - 107 NeuroTrace Green (Invitrogen, 1:300)
 - Invert to mix and pipet 6ml per 5ml tube

Day 10 (Wed)

- Secondary Antibody (Goat Alexa647 Anti-Rabbit) and NeuroTrace Green, day 2 of 3

- Secondary antibody solution with Goat Alexa647 Anti-Rabbit and NeuroTrace Green O/N @ 37C, on nutator
 - 32 ml Secondary Buffer
 - 64 ul Goat Alexa647 Anti-Rabbit (Invitrogen, 1:500)
 - 107 NeuroTrace Green (Invitrogen, 1:300)
 - Invert to mix and pipet 6ml per 5ml tube

Day 11 (Thur)

- Secondary Antibody (Goat Alexa647 Anti-Rabbit) and NeuroTrace Green, day 3 of 3

- Secondary antibody solution with Goat Alexa647 Anti-Rabbit and NeuroTrace Green O/N @ 37C, on nutator
 - 32 ml Secondary Buffer
 - 64 ul Goat Alexa647 Anti-Rabbit (Invitrogen, 1:500)
 - 107 NeuroTrace Green (Invitrogen, 1:300)
 - Invert to mix and pipet 6ml per 5ml tube

Day 12 (Fri)

- Washing

- Transfer to new 5ml tube
- PTwH for 10min @ RT, on nutator (protect from light)
- PTwH for 10min @ RT, on nutator (protect from light)
- PTwH for 10min @ RT, on nutator (protect from light)
- PTwH for 60min @ RT, on nutator (protect from light)
- PTwH for 120min @ RT, on nutator (protect from light)
- PTwH for 120min @ RT, on nutator (protect from light)
- PTwH for 3 days (over weekend) @ RT, on nutator (protect from light)

Day 15 (Mon)

- Dehydration for Clearing

- Transfer to new 5ml tube
- 20% MeOH for 60min @ RT, on nutator (protect from light)
- 40% MeOH for 60min @ RT, on nutator (protect from light)
- 60% MeOH for 60min @ RT, on nutator (protect from light)
- 80% MeOH for 60min @ RT, on nutator (protect from light)
- 100% MeOH for 60min @ RT, on nutator (protect from light)

- Transfer to new 5ml tube
- 100% MeOH for 60min @ RT, on nutator (protect from light)

- Transfer to new 5ml tube
- 66% DCM + 33% MeOH O/N @ RT, on nutator (protect from light)
 - 24ml DCM (draw out using pipetting needle and syringe and only use conical tubes)
 - 12ml 100% MeOH
 - Vortex and pipet 6ml per 5ml tube

Day 16 (Tues)

- Dehydration for Clearing

- Transfer to new 5ml tube
- 100% DCM for 15min @ RT, on nutator (protect from light)
- 100% DCM for 15min @ RT, on nutator (protect from light)
 - Draw out DCM using pipetting needle and syringe and only use conical tubes

- Transfer individual sections (in order of anterior to posterior) to new 1.5ml Eppendorf tubes (1 section/tube)
- 100% DBE O/N (but can be as short as 2 hours) @ RT, keep tubes on side (protect from light)

Day 17 (Wed)

- Mount and Seal Tissue on Slides

- Mount cleared tissue onto glass slides in new DBE (protect from light)
- Mount using 500um silicone spacers (and 100um glass coverslip spacers, if necessary; protect from light)
- Seal corners first with silicone and place weights on slides to prevent shifting (protect from light)
- Remove excess DBE and seal edges with silicone (protect from light)