Lab Recipes
General Stock Solutions

Note: Recipes have been calculated using weight by volume, meaning that the solvent is added up to a certain volume after accounting for the volume of the solute.

For all recipes: Label all solutions. Include your name, the date (mm/dd/yy), and any other applicable information (e.g. pH).

0.9% Normal Saline Solution: **1 L**

- *NaCl* *9.0 g*
- *MilliQ water (H_2O) to* *1 L*

Filter into a 1 L bottle

10x PBS (100 mM) – pH 6.8 **1 L**

- *Sodium chloride (NaCl)* *80 g*
- *Potassium chloride (KCl)* *2 g*
- *Sodium phosphate dibasic anhydrous (Na_2HPO_4)* *14.4 g*
- *Potassium phosphate monobasic anhydrous (KH_2PO_4)* *2.4 g*
- *MilliQ water (H_2O) to* *1 L*
- *Sodium hydroxide (NaOH)* *as needed*
- *Hydrogen chloride (HCl)* *as needed*

Add everything to 800 mL of water.
Make sure mixture is completely dissolved before measuring pH.
Adjust pH with NaOH and HCL to bring it to 6.75 – 6.85 at room temperature.
Add water up to 1 L.

1x PBS (10mM) – pH 7.4 **1 L**

- *10x PBS* *100 mL*
- *MilliQ water (H_2O)* *900 mL*
- *Sodium hydroxide (NaOH)* *as needed*
- *Hydrogen chloride (HCl)* *as needed*

Adjust pH with NaOH and HCl to bring it to 7.4 at room temperature.

0.4% PBS-Tx **500 mL**

- *Triton X-100* *2 mL*
- *1x PBS* *498 mL*

Gently upturn the bottle a few times to thoroughly mix the solution without creating excess bubbles.

10% PBS Azide **100 mL**

- *Sodium azide (NaN_3)* *10 g*
- *1x PBS to* *100 mL*

1% PBS Azide	**500 mL**
– *10% PBS azide*	*5 mL*
– *1x PBS*	*495 mL*

30% Sucrose Solution	**20 mL**
– *Sucrose ($C_{12}H_{22}O_{11}$)*	*6 g*
– *1x PBS to*	*20 mL*

Sodium Hydroxide (1.0 M)	**100 mL**	**250 mL**
– *Sodium hydroxide (NaOH)*	*4.0 g*	*10 g*
– *MilliQ water (H_2O) to*	*100 mL*	*250 mL*

CAUTION: This is an exothermic reaction.
Slowly add NaOH to 80 mL (or 200 mL) of water, wait until solution cools.
Add water up to final volume.

4% Paraformaldehyde Fixative (0.1 M) – pH 7.4	**400 mL**	**800 mL**
– *Disodium phosphate (Na_2HPO_4)*	*4.36 g*	*8.72 g*
– *Monosodium phosphate (NaH_2PO_4)*	*1.28 g*	*2.56 g*
– *Paraformaldehyde powder*	*16.0 g*	*32.0 g*
– *MilliQ water (H_2O) to*	*400 mL*	*800 g*
– *Sodium hydroxide (NaOH)*	*as needed*	*as needed*
– *Hydrogen chloride (HCl)*	*as needed*	*as needed*

Note: Only use items marked with "F" when making fixative. Wear gloves!

1. Add 350 mL (or 750 mL) of water, magnetic stir bar, and thermometer to a 1 L (or 2 L) beaker.
2. Using a hot plate in the fume hood, heat water to ~68°C.
   a. Make sure temperature does not exceed 70°C.
3. Turn off heat element and remove thermometer.
4. Add paraformaldehyde powder over 10 minutes. Stir vigorously to dissolve.
5. Add drops of NaOH until the solution is clear when settled.
6. Add Na_2HPO_4 and NaH_2PO_4 to solution.
7. Cool solution to room temperature before adjusting the pH with HCL. Final pH should be 7.4 at room temperature.
8. Add water up to appropriate final volume.
9. Filter into a 500 mL (or 1 L) bottle and store in the fridge at 4°C.

Lab Recipes
Immunohistochemistry Solutions

Bleach

(50% methanol & 1% hydrogen peroxide in PBS)	**18 mL**
– *30% Hydrogen peroxide (H_2O_2)*	*700 µL*
– *Methanol (CH_3OH)*	*9 mL*
– *1x PBS*	*9 mL*

Streptavidin Peroxidase Medium (1:1,000)	**20 mL**
– *SA-HRP (Molecular Probes (1mg/ml))*	*20 µL*
– *0.4% PBS-Tx*	*20 mL*

Diaminobenzidine Peroxidase Reaction Medium (for <u>BROWN</u> reaction product)	**50 mL**	**100 mL**
– *30% Hydrogen peroxide (H_2O_2)*	*7.5 µl*	*15 µl*
– *DAB (Sigma)*	*12.5 mg*	*25 mg*
– *1x PBS*	*50 mL*	*100 mL*

Diaminobenzidine peroxidase reaction medium (for <u>BLACK</u> reaction product)	**50 mL**	**100 mL**
– *0.5% Cobalt(II) chloride ($CoCl_2$)*	*1.5 mL*	*3 mL*
– *30% Hydrogen peroxide (H_2O_2)*	*7.5 µl*	*15 µl*
– *DAB (Sigma)*	*12.5 mg*	*25 mg*
– *1x PBS*	*48.5 mL*	*97 mL*

Anti-CTB (1:30,000) Incubating Medium *WITHOUT* Sodium Azide (for incubations overnight at 4°C)	**1 mL**
– *Anti-choleragenoid stock solution 1:1,000*	*33 µl*
– *Normal rabbit serum (Sigma)*	*25 µl*
– *0.4% PBS-Tx*	*942 µl*

Anti-CTB (1:30,000) Incubating Medium *WITH* 0.1% Sodium Azide (for incubations at RT or at 4°C)	**1 mL**
– *Anti-choleragenoid stock solution 1:1,000*	*33 µl*
– *Normal rabbit serum (Sigma)*	*25 µl*
– *10% Sodium azide (NaN_3)*	*10 µl*
– *0.4% PBS-Tx*	*932 µl*

Biotinylated anti-goat medium (1:200)	**20 mL**
– *Biotinylated rabbit anti-goat (Sigma)*	*100 µl*
– *0.4% PBS-Tx*	*20 mL*

Lab Recipes
Nissl Counterstain Solutions

1% Thionin Stock Solution **200 mL**

- *Thionin* *2 g*
- *MilliQ water (H_2O) to* *200 mL*

Bring water to a boil.
Turn off heat. Add thionin while stirring.
Allow solution to stir o/n.
Filter and store in a brown glass bottle, protected from light.

Acetic Acid Solution (1M) **500 mL**

- *Glacial acetic acid* *28.5 mL*
- *MilliQ water (H_2O)* *471.5 mL*

Mix and store in the freezer at -20°C.

Sodium Acetate Solution (1M) **1 L**

- *Hydrous sodium acetate (1M sodium acetate)* *136.08 g*
- *MilliQ water (H_2O) to* *1 L*

Add sodium acetate to 800 mL water.
Stir and bring to a final volume of 1 L.
Store in 500 mL aliquots in the freezer at -20°C.

Thionin Buffer Solution - pH 4.4 **540 mL**

- *1M Acetic acid* *72 mL*
- *1M Sodium acetate* *48 mL*
- *MilliQ water (H_2O)* *420 mL*

Check pH (~4.4). Filter.

0.1% Thionin Stain **200 mL**

- *1% Thionin stock solution* *20 mL*
- *Thionin stain buffer solution* *180 mL*