MABT for *in situ* Hybridization

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| **Reagents** | **Volume Added** | **Final Concentration** |
| Maleic Acid (M.W. 116.07) | 4.35 g | 150 mM |
| NaCl (F.W. 58.44) | 1.46 g | 100 mM |

1. Collect items as follows:

Bottle of **RNase-free H2O**

250 mL graduated cylinder

300 mL glass beaker

Weigh boats

**Maleic acid** (M.W. 116.07)

**NaOH pellets**

50 mL conical vial of **NaOH, 1M**

Bottle of **HCl** located in fume hood

P200 pipette w/ adjoining sterile-filter tips

**NaCl** (F.W. 58.44)

50 mL bottle of undiluted **Tween-20**

P1000 pipette with sterile-filter tips

Sterile filter 250 mL bottle

Magnetic stir bar

2. Add 200 ml of RNase-free H2O to the 300 mL glass beaker.

3. Weigh out 4.35 g of maleic acid on a weigh boat and pour its contents into the 300mL glass beaker. Use the probe in order to record its pH. It should read approximately 1.65 pH. Stir with magnetic stir bar.

4. Adjust the pH w/ NaOH pellet-wise to 7.5. It will take approximately 25 pellets. Add them slowly and allow them to suspend into solution before adding more. If the pH reaches a value > 7.5, add HCl dropwise to the solution (10 µl ≈ .1 pH decrease).

5. Add 1.46 g of NaCl to the 300 mL glass beaker.

6. Using a P1000, slowly (over the course of several minutes) pull up 250 µl of Tween-20 and add it to the 300 mL glass beaker. (*Be sure not to incorporate bubbles or residual Tween-20 dripping off the pipette tip.)*

7. Add the contents of the 300 mL glass beaker to the 250 mL graduated cylinder. Top off the solution with RNase-free H2O for an end volume of 250mL.

8. Add the contents to a sterile filter 250 mL bottle. Pull the contents through the sterile filter using a vacuum. Wrap the bottle in aluminum foil, label it “MABT’ and store in the 4°C fridge.