

COM Procedure Handout

Time Required: 75 minutes plus waiting 3 days

Chemicals

calcium chloride dihydrate
sodium oxalate
sodium chloride

Inhibitors

albumin from bovine serum
human transferrin
chondroitin sulfate A sodium salt from bovine trachea
sodium citrate dehydrate
dimethyl hydroxyglutaric acid

Procedure

1. Make 10 mM stock solutions of calcium chloride and sodium oxalate using round-bottom flasks. Make stock solutions of the inhibitors at concentrations of 2 g/liter.

10 mmol	1 L	1 mol	147.01 g
1 L	1000 mmol		mol
=1.4701 g of calcium chloride dihydrate in 1 L			

10 mM	1 L	1 mol	134.00 g
	1000 mmol		mol
=1.34 g of sodium oxalate in 1 L			

2 g	1 L	25 ml	
1 L	1000 ml		
=0.05 g of inhibitor in 25 ml			

2. Measure out NaCl and add to deionized water in a 20-ml vial. The amount of deionized water to add is the amount that will not be from the stock solutions of calcium chloride, sodium oxalate or inhibitor to make a 10 ml solution (8.35 ml with inhibitor or 8.6 ml without inhibitor). Stir with stir bar on magnetic pad to dissolve all of the salt.

150 NaCl concentration

150 mmol	1 L	10 ml	1 mol	58.44 g
1 L	1000 ml	1000 mmol		mol
=0.08766 g of sodium chloride for 10 ml crystallization solution				

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3. Add 10 mM stock solution of calcium chloride to a 20-ml vial to create 0.7 mM solution calcium chloride.

0.7 mM calcium chloride concentration

0.7 mmol	1 L	10 ml	1 L	1000 ml
1 L	1000 ml		10 mmol	1 L

=0.7 mL of stock solutions for 10 mL crystallization solution

4. Allow solution to stir for 1 minute and place into 60 °C oven for 1 hour, capped.
5. Use a diamond cutter or file to cut a microscope slide into tiny squares that can fit laying down at the bottom of the vial (approximately 1 cm × 1 cm). Wash the glass squares with acetone and let them dry on a kimwipe. Use tweezers; do not touch them with your hands.
6. Remove the vial from the oven and place it back onto the stir plate. Add inhibitor solution to produce 50 µg/ml concentration of inhibitor in 10 ml.

50 µg/ml of inhibitor

50 µg	10 ml	ml
ml		2000 µg

=0.25 ml inhibitor

7. Add 10 mM sodium oxalate stock solution to the vial to produce 0.7 mM sodium oxalate solution in the vial. Let the solution stir for about a minute.
8. Remove the stir bar using a magnetic stick. Place 1 square glass piece at the bottom of the vial. Cap the vial and place the vial into the 60 °C oven for 3 days.
9. After three days, remove the vial from the oven. Using tweezers, remove the square glass piece from the vial, rinse gently with deionized water and let it air dry overnight in a dry petri dish.