**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

1. 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

1. 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

1. Allow solution to stir for 1 minute and place into 60 °C oven for 1 hour, capped.
2. 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.
3. 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 ug/ml of inhibitor

|  |  |  |
| --- | --- | --- |
| 50 μg | 10 ml | ml |
| ml |  | 2000 μg |

=0.25 ml inhibitor

1. 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.
2. 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.
3. 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.