GLUCURONIC ACID ASSAY

Reagents:
A: 12.5mM sodium tetraborate decahydrate (Borax) in sulfuric acid
    = 4.7671 g in 1L volumetric flask of sulfuric acid
B: 0.5% NaOH + 0.15% m-hydroxydiphenyl (phenyl phenol)
    =Dissolve 0.5 g NaOH in approx. 80ml distilled water.  Add 0.15 g m-Hdp and
    stir with stir bar to dissolve.
    Transfer to 100 ml volumetric flask and add d-water to 100ml line on flask
Blank: Dissolve 0.5g NaOH in 80 ml d-water.  Transfer for 100 ml volumetric flask and
    add d-water to fill line on flask

GA standards:
STOCK: 0.01 M GA stock:
    =0.1941 g GA in 100 ml volumetric flask with d-water
Dilute stock to make 250uM solution for standard curve:
    Add 5.0 ml of 0.01M GA stock in 200 ml volumetric flask and fill with d-water to
    fill line on flask

PREPARE:
1. Ice bath
2. Boiling water bath
3. Labeled 5 ml glass test tubes in triplicate for each sample and for each standard
4. Cuvettes
5. Extra ice
6. Spectrophotometer at absorbance of 520nm

GA STANDARDS
<table>
<thead>
<tr>
<th>mL d-water</th>
<th>mL 250uM GA solution</th>
<th>nmole/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.0</td>
<td>250</td>
</tr>
<tr>
<td>1.0</td>
<td>4.0</td>
<td>200</td>
</tr>
<tr>
<td>3.0</td>
<td>2.0</td>
<td>100</td>
</tr>
<tr>
<td>4.0</td>
<td>1.0</td>
<td>50</td>
</tr>
<tr>
<td>4.5</td>
<td>0.5</td>
<td>25</td>
</tr>
<tr>
<td>5.0</td>
<td>0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

PREPARE URINE SAMPLES
Dilute urine samples as follows:
Control: add 3960ul of d-water to each test tube + 40 ul of vortexed urine = 1 part urine
to 100 part water

Toxic: add 9960ul of d-water to each test tube + 40 ul of vortexed urine = 1 part urine to
250 part water
METHODS:
1. Add 0.5 ml of standard and diluted sample in triplicate to each test tube and place test tube in water bath. Vortex urine or standard before adding to t.t.
2. Add 3.0 ml of Reagent A, vortex well and return to ice
   1. pour reagent A into large beaker and use repeat pipettor:
   2. zero pipettor
   3. select 50 ml syringe with adapter and repeat function
   4. select for 3.0 ml to dispense
   5. Place syringe in reagent A solution
   6. Suck up solution slowly to about 1/2 way
   7. Turn pipettor over and purge using down arrow until see all air goes out of pipette, don’t let acid go all over.
   8. Then place pipettor back in solution and slowly suck up rest of way
   9. Expel first 3.0 ml into reagent beaker using blue button
10. Then pipette 3.0 ml into each tube – you can do about 15 at a time. Don’t pipette out all solution before you refill, it will start to drip and will be harder to fill
11. After 15 tubes, suck up more solution slowly
3. Place lids or marbles on test tubes
4. Place test tube in boiling water bath for 5 minutes
5. Remove test tubes from boiling bath and place on ice bath
6. One set of test tubes gets 50 ul of 0.5% NaOH as blank
7. Two sets get 50ul of reagent B
8. Vortex all tubes after adding either NaOH or reagent B and remove from ice bath
9. Let sit at room temp for 10 minutes
10. Ready to read on spec

SPECTROPHOTOMETER
1. Run standards first
2. Place 0 standard blank in cuvet and place in spec and zero spec
3. Then place your duplicates of 0 standard and record absorbance
4. Always zero machine with blank for each sample before reading the sample