BF media from powder – protocol

For 1 L:

1. Put 500 ml ddH2O into a beaker
2. Add:
   1. 1 pkg of IMDM powder (#12200036 Thermo) = 17.7g
   2. 3.025g Sodium Bicarbonate
   3. 184 mg L-cystein
   4. 29 mg Bathocuproinedisulfonic acid or 29.9 mg Bathocuproinedisulfonic acid hydrate
3. Mix 1 hour
4. Meanwhile – 136 mg hypoxanthine -> 19.8 ml H20 + 200 ul 10 N NaOH -> dissolve on shaker
5. Add Hypoxanthine
6. Measure pH – should be really close to 7.4
7. Bring volume up to 880 ml.
8. Measure pH – bring to 7.4 if it changed.
9. Mix 15 min
10. Filter sterilize
11. Add
    1. 12.5 ml Sodium pyruvate solution
    2. 10 ml Penstrep
    3. 100 ml FBS
12. For working stock: add 100 ul of 100 mM B-Me to 100 ml media
13. Drug concentrations
    1. Stock working conc ul/ 100 ml media
    2. G418 100 mg/ml 2 .5 ug/ml 2.5
    3. Hygromycin B 100 mg/ml 1.5 ug/ml 1.5
    4. Phleomycin 10 mg/ml 2.5 ug/ml 25
    5. Puromycin 10 mg/ml 0.1 ug/ml 1
    6. Blasticidin 10 mg/ml 5 ug/ml 50