Complete GMEM-10

Add the following in order given, using aseptic technique:

- 500 ml Glasgow modified Eagle medium (GMEM) without tryptose phosphate broth (Life Technologies, but made without glutamine; must be made to order as it is not a stock item)
- 5 ml 100× nonessential amino acids (Life Technologies)
- 5 ml glutamate + asparagine (G+A)
  o Recipe:
    ▪ 600 mg L-glutamic acid (Sigma)
    ▪ 600 mg L-asparagine (Sigma)
    ▪ H2O to 100 ml
    ▪ Filter sterilize using a 2-µm filter and store at 4°C
- 5 ml 100 mM sodium pyruvate (Life Technologies)
- 10 ml 50× nucleoside mix (see recipe)
  o Recipe:
    ▪ 35 mg adenosine (Sigma)
    ▪ 35 mg guanosine (Sigma)
    ▪ 35 mg cytidine (Sigma)
    ▪ 35 mg uridine (Sigma)
    ▪ 35 mg thymidine (Sigma)
    ▪ H2O to 100 ml
    ▪ Filter sterilize and store at −20° in 10-ml aliquots
- 50 ml dialyzed FBS (see recipe)
  o Recipe:
    ▪ Purchase dialyzed FBS from commercial supplier (e.g., Life Technologies or J.R.H. Biosciences) or prepare as follows:
      1. Heat inactivate FBS at 56°C for 60 min.
      2. Soak Spectrapor dialysis tubing (MWCO 6000 to 8000) in PBS (APPENDIX 2). Remove, rinse tubing, clip one end closed, and fill with the heat-inactivated FBS.
      3. Dialyze (also see APPENDIX 3C) 6 to 8 hr in cold room against PBS. Change dialysis solution at least once.
      4. Filter sterilize using a 0.02-mm filter and store frozen (−20°C) in 50-ml aliquots.
- 5 ml of 5000 U/ml penicillin/streptomycin (Life Technologies)

It is essential to use dialyzed FBS when performing GS selection because serum contains significant amounts of glutamine.