Media: DMEM high glucose liquid media (500 ml) [Biological Industries, #01-055-1A]
Fetal Calf Serum (Biological Industries): 10%
PSA (antibiotics- penicillin, streptomycin, and amphotericin B; 1% [Sartorius, 03-033-1B]
Gln (amino acid- glutamine; Biological Industries): 1%

Method:

1. Thaw two aliquots of 25 ml of FCS, 1 aliquot of 5ml PSA, and 1 aliquot of 5 ml Gln (stored in the freezer, room- tissue culture) at 37 °C in a water bath.
2. Warm the cold 500 ml liquid DMEM high glucose (stored at 4 °C, room: 3-518) also in a water bath.
3. Once the reagents are thawed clean the surfaces of the bottles and tubes by spraying 70% alcohol, wipe-dry, and transfer them to the working Hood.
4. To the 500 ml DMEM media transfer a total of 50 ml FCS using sterile 25 ml pipette, 5 ml PSA, and 5 ml Gln using 5 ml pipette, mix well by swirling the bottle.
5. Write on the bottle with a thick marker: COMPLETE MEDIUM, preparation date, your name, +10% FCS, + 1% PSA, and + 1% Gln and store it at 4 °C for further use. The complete medium should be used for up to about one month.