

## Reagent

Amino acid free Dulbecco's modified Eagle medium powder (USBiological, D9800-13)

Glucose powder (Sigma, SLBN1246V)

Sodium bicarbonate powder (Sigma, S6014)

MEM Amino acid solution 50X (gibco, 11130051 or Hyclone SH30598.01)

Glutamine, 100X, 0.2M (UCSF CCF, CCFGB002-15C702)

## Amino Acid free DMEM solution(FBS free)

1. Dissolve 4.16g amino acid free Dulbecco's modified Eagle medium powder and 1.75 g glucose in 450ml sterilized ddH<sub>2</sub>O, mix thoroughly until completely solubilized.
2. Add **approximately** 1g sodium bicarbonate powder adjusting PH to 7.0.
3. Add additional water to bring the solution to 500ml.
4. Filter-sterilize using a 0.22 micron membrane filter. Aliquot into sterile containers.
5. Add 5ml 100X penicillin-streptomycin solution.

## Amino Acid Add back solution

1. Add amino acid and glutamine into amino acid free DMEM solution. Dilute 50X amino acid solution and 100X Glutamine solution to final concentration of 1X.
2. Add sodium bicarbonate powder adjusting PH to 7.0-7.5(50X amino Acid solution is super acid).
3. Filter-sterilize using a 0.22 micron membrane filter. Aliquot into sterile containers.