

DMEM MEDIA PREPARATION

Materials:

Dulbecco's Modified Eagle Medium

with high glucose

with L-glutamine

with pyridoxine hydrochloride

without sodium pyruvate

without sodium bicarbonate

Invitrogen/Gibco (cat# 12100-046 / packs of 10x1-liter packages)

dH₂O

Sodium bicarbonate (NaCO₃)

Sterile cell culture (45mm neck) bottle

Millipore GP Express PLUS vacuum 0.22µm filter top 45mm neck:

150ml SCGPTO1RE

500ml SCGPTO5RE

To prepare 1x liquid medium:

1. Measure out 5% less sterile dH₂O than desired total volume of media.
2. Add powdered media to room temp sterile dH₂O with gentle stirring (do not heat).
3. Rinse out inside of package to remove all traces of powder and add to media.
4. Add 3.7g of NaCO₃ per liter of media.
5. Add dH₂O to desired volume. Stir until dissolved (do not over mix).
6. The final pH of the media should be pH 7.4. Adjust pH of media to 0.2-0.3 below the desired working pH using 1N NaOH or 1N HCl (add slowly with stirring). The pH units will increase 0.1-0.3 upon filtration. After pH has been adjusted, keep container closed until media is filtered.
7. Sterilize immediately by using a Millipore vacuum 0.22µm filter top.
8. Store media at 4°C in the dark for up to 12 months*.

*according to Invitrogen technical service, the powder media is good for up to 36 months if kept at 4°C and in a dark and dry location. Once the liquid media is prepared, it is good for at least one year if stored at 4°C in the dark.