

RECIPE BOOK

Agar Coated & Injection Dishes

1.2% Agarose in Embryo Media (don't use LMP Agarose)

DEPC Water

CAUTION: DEPC is carcinogenic. Wear gloves. Stored at 4C.

0.1% Diethyl Pyrocarbonate in water (500ul DEPC in 500mls H₂O)

Shake at 37C overnight.

Autoclave

0.5M EDTA pH 8.0

per liter: 186.1g disodium EDTA-2H₂O

800 ml distilled water

stir vigorously (it takes forever! heat OR add NaOH to help into solution, but don't heat and add NaOH as NaOH is volatile)

pH to 8.0 with about 10g of NaOH pellets

Embryo Media

per liter: 50mls 20X embryo media

2mls 500X NaHCO₃

fill to 1Liter with distilled water

if adding penicillin- streptomycin solution, add 10mls/ liter, and filter (0.22um CN).

made fresh each week and stored at room temperature

20X Embryo Media

per liter: 17.5g NaCl

0.75g KCl

2.9g CaCl-2H₂O

add distilled water to 800mls

0.41g KH₂PO₄

0.142g Na₂HPO₄ anhydrous

4.9g MgSO₄-7H₂O

add distilled water to a liter. vacuum filter. store at 4°C for months

8% Paraformaldehyde Fixative

CAREFUL: PFA is a nasty toxin. When weighing out the solid, wear a mask and gloves. Use the solutions in the hood and wear gloves.

per 100mls: add 8g of Paraformaldehyde to 100mls sterile water

stir into solution (add 8 drops of 1N NaOH to help into solution)

gravity filter through a fine pore filter

store at 4°C. good for one week!

working stock is: 4% Fix and 1X PBS. make fresh for every use.

LB Broth

per liter: 10g Bacto-tryptone
5g Bacto-yeast extract
10g NaCl

pH to 7.0 with 100ul 10N NaOH. autoclave.

if making plates: add 16g Bacto-Agar per Liter LB. after autoclaving, let agar cool to a comfortable touch. add drug and pour. 1 Liter makes 2 sleeves of plates. Lab drugs stocks are at 1000X.

1M MgCl₂

per liter: 203.3g MgCl₂ · 6H₂O
800mls distilled water
autoclave

5M NaCl

per 500mls: FW= 58.44

add 146.1g to 450mls distilled water. heat and stir into solution. bring the final volume up to 500mls. autoclave.

10X PBS

per liter: 80g NaCl
2g KCl
14.4g Na₂HPO₄ (dibasic anhydrous)
2.4g KH₂PO₄ (monobasic anhydrous)
800mls distilled water
pH to 7.4 . autoclave. store at room temperature.

Pronase E

2mg/ml in embryo media
store at -20C as 0.5ml aliquots

500X Sodium Bicarbonate

0.35g NaHCO₃ in 10mls water
store at 4°C. Make fresh every week.

Sterile water

fill bottles with distilled water and autoclave

20X SSC

per 500mls: 87.65g NaCl
44.1g Sodium Citrate
start with 350mls distilled water. stir. add salts. let sit and bring up to volume in a graduated cylinder.

1M Tris

per liter: 121.1g Tris Base
800ml distilled water
adjust the pH with concentrated HCl

10X TBE

per liter: 108g Tris Base
55g Boric Acid
40ml 0.5M EDTA

start with 800mls distilled water. add all ingredients. when in solution, dump into the carboy and fill to the 20Liter line with distilled water for a final concentration of 0.5X.