

4.9 Reagents and Media

Media used in tissue culture must be sterile. This may be accomplished by mixing all the ingredients and filtering with a 0.2 μm filter, or, by mixing of the stable ingredients, autoclaving, and then aseptically adding the labile ingredients such as glutamine, serum, and antibiotics.

A. Sample Dilution Medium - Hanks Balanced Salt Solution (HBSS)

10X HBSS	100.0 mL
Tissue culture grade water	895.3 mL
NaHCO ₃ (7.5%)	4.7 mL

Mix and ensure sterility.

B. Antibiotic Incubation Medium (Anti-Inc) Made with HBSS for Sample Disinfection

10X HBSS	100.0 mL
Tissue culture grade water	575.0 mL
NaHCO ₃ (7.5%)	5.0 mL
Penicillin/streptomycin	160.0 mL
Penicillin G (10,000 units/mL)	
Streptomycin sulfate (10,000 $\mu\text{g}/\text{mL}$)	
Fungizone	160.0 mL
250 $\mu\text{g}/\text{mL}$ amphotericin B	
205 $\mu\text{g}/\text{mL}$ desoxycholate	

Mix and ensure sterility. Store at 4° C. Equal volumes of sample and anti-inc are mixed together for sample disinfection.

C. Antibiotic Incubation Medium (Anti-Inc) Made with Minimum Essential Medium (MEM-0) for Sample Disinfection

10X MEM (Eagles Modified Medium)	100.0 mL
Tissue culture grade water	540.0 mL
L-Glutamine (200 mM)	10.0 mL
NaHCO ₃ (7.5%)	30.0 mL
Tryptose phosphate broth	100.0 mL
Penicillin/streptomycin	160.0 mL
Penicillin G (10,000 units/mL)	
Streptomycin sulfate (10,000 $\mu\text{g}/\text{mL}$)	
Fungizone	160.0 mL

250 µg/mL amphotericin B
205 µg/mL desoxycholate

Mix and ensure sterility. This may be stored frozen for approximately three months. Avoid freeze-thaw cycles; thaw tubes immediately prior to use. Equal volumes of sample and anti-inc are mixed together for sample disinfection.

D. Versene (EDTA) (1:5000)

NaCl	8.0 g
KHPO ₄	0.2 g
KCl	0.2 g
Na ₂ HPO ₄	1.15 g
Disodium versenate (EDTA)	0.2 g
Phenol red (0.5% solution)	2.0 mL
Tissue culture grade water	to 1000 mL

Autoclave and store at room temperature.

E. Trypsin-Versene (EDTA)

Trypsin (2.5% solution)	20 mL
Versene (EDTA) (1:5000)	480 mL

Store at -20° C.

F. MEM-5/Hepes (Tissue Culture Medium for all Cell Lines Except SHK-1 and ASK)

Tissue culture grade water	810.0 mL
10X MEM	100.0 ml
Fetal bovine serum	50.0 mL
Sodium bicarbonate (7.5% solution)	10.0 mL
L-Glutamine (200 mM)	10.0 mL
Hepes buffer (1M)	15.0 mL
NaOH (1M)	5.0 mL
NaOH or HCL - as needed to adjust pH to 7.2-7.6	

If antimicrobials are included, use 796.0 mL of water above instead of 810.0 and add:

Gentamicin (50 mg/mL)	4.0 mL
Fungizone	10.0 mL
250 µg/mL amphotericin B	
205 µg/mL desoxycholate	

Mix and ensure sterility. Store at 4° C.

G. MEM-10/Hepes (Tissue Culture Medium for all Cell Lines Except SHK-1 and ASK)

10X MEM	100.0 mL
Tissue culture grade water	760.0 mL
Fetal bovine serum	100.0 mL
Sodium bicarbonate (7.5% solution)	10.0 mL
L-Glutamine (200 mM)	10.0 mL
Hepes buffer (1M)	15.0 mL
NaOH (1M)	5.0 mL
NaOH or HCL - as needed to adjust pH to 7.2-7.6	

Mix and ensure sterility. Store at 4° C.

H. Leibovitz's L-15 (Tissue Culture Medium for SHK-1 and ASK Cell Lines)

1X L-15 with 0.3g/L L-glutamine	1000.0 mL
Fetal bovine serum (5%)	50.0 mL
Gentamicin (50 mg/mL)	1.0 mL
2-mercaptoethanol (0.055 M)	0.7 mL
NaOH or HCL - as needed to adjust pH to 7.2-7.6	

Mix and ensure sterility. Store at 4° C.