

## Mack Growth Media (GM) and HEPES Buffer

### General Notes

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- Mack cells should be cultured in vented flasks at 27°C without CO<sub>2</sub>
- Mack GM and HEPES solution should be stored at 4°C

### Materials

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- Leibovitz's L-15 Medium
- Antibiotic-Antimycotic (100X)
- HEPES (1 M, pH 7.4)
  - HEPES solids used to prepare this (protocol included)
- Fibroblast Growth Factor 2 (FGF-2, recombinant human) (5 ng/μL)
- 0.22 μm Filter Top with Bottle (Nalgene™ Rapid-Flow™ Sterile
- Single Use Vacuum Filter Units, 500 mL)
- Gibco Qualified Fetal Bovine Serum (FBS)
- Clean beaker
- Stir bar
- Distilled water
- Hydrochloric acid (HCl)
- Sodium hydroxide (NaOH)

### Method

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1. Optional: Prepare 100 mL 1M HEPES Buffer
  - a. To a clean beaker, add ~60 mL of distilled water
  - b. Add 23.84 of HEPES solids
  - c. Place a stir bar in beaker and stir until HEPES solids are in solution
  - d. pH adjust the solution to pH7.4 with HCl and NaOH
    - i. The initial pH will be below pH7.4 so NaOH will be used predominantly
  - e. Pour solution into a 100 mL graduated cylinder
  - f. Add distilled water until the total solution volume is 100 mL
  - g. Sterile filter solution through 0.22 μm filter
  - h. Store at 4°C

2. Combine the following components and volumes to prepare 500 mL of Mack GM

Component	Stock Concentration	Quantity	Media Concentration
Leibovitz's L-15 Medium	n/a	385 mL	n/a
Antibiotic-Antimycotic (100X)	100X	5 ml	1X
HEPES (1M)	1000 mM	10 mL	20 mM
Fibroblast Growth Factor (FGF-2) (5 ng/μL)	5000 ng/mL	0.1 mL	1 ng/mL
Gibco Qualified Fetal Bovine Serum (FBS)	100%	100 mL	20%

3. Sterile filter Mack GM through 0.22  $\mu\text{m}$  filter
4. Store at 4°C