

**Buffer Preparations**  
(April 2020)

**Introduction**

This protocol describes the preparation of HEPES buffer at pH 7.8.

**Definitions**

HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)

M: Molar

**Reagents and Materials**

- Endotoxin-Free Water  
Lot #: \_\_\_\_\_  
Date Opened: \_\_\_\_\_  
*Manufacturer: HyClone*
- 1 mL 1 M HEPES Buffer Solution  
Lot#: \_\_\_\_\_  
Date Opened: \_\_\_\_\_  
*Manufacture: Sigman*
- 1N NaOH  
Manufacture date: \_\_\_\_\_  
Lot#: \_\_\_\_\_  
*Manufacturer: In house*
- pH 4.00 Standard Buffer  
Lot#: \_\_\_\_\_  
*Manufacturer: Fisher Scientific*
- pH 7.00 Standard Buffer  
Lot#: \_\_\_\_\_  
*Manufacturer: Fisher Scientific*
- pH 10.00 Standard Buffer  
Lot#: \_\_\_\_\_  
*Manufacturer: Fisher Scientific*
- Heated Stir Plate  
*Manufacturer: Fisher Scientific*
- HPLC grade water  
*Manufacturer: In house*
- Serological Pipet

*Manufacturer:* Fisher Scientific

- Serological Pipet Tips  
25 ml  
*Manufacturer:* Fisher Scientific
- Pipetmen  
P 1000  
*Manufacturer:* Fisher Scientific
- Pipetmen Tips  
P 1000  
*Manufacturer:* Fisher Scientific

### **Instrumentation**

Fisher Accumet Basic AB15 Plus pH Meter with pH probe

Probe SN: \_\_\_\_\_

*Manufacturer:* Fisher Scientific

### **Protocol**

1. Measure out 99 mL of Endotoxin-free water into clean, dry, 100 mL glass bottle with clean cap and magnetic stir bar
2. Add 1 mL of 1 M HEPES Buffer to bottle from step 1
3. Standardize pH probe using three pH standard buffers, rinsing probe with HPLC grade water between samples, wipe dry with Kim Wipe;  
Slope \_\_\_\_\_
4. Rinse and dry pH probe, measure pH of buffer
5. Adjust pH up to 7.8 with 1 N NaOH, dropwise;  
Final pH \_\_\_\_\_
6. Tightly cap glass bottle, label, store at 4°C; Label \_\_\_\_\_