

# Stock Solution



TD-S Revision 2.0

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## 1M HEPES-Na Buffer - 1 L

### Instructions

1. Dissolve 260.29 g of HEPES, Sodium Salt ([HEPES, Sodium Salt, GoldBio Catalog # H-401](#) [CAS 75277-39-3, mw. = 260.29]) in 750 mL of dH<sub>2</sub>O.
2. Adjust to desired pH using concentrated HCl. Note: This method will produce about 0.25M-0.9M NaCl in the concentrated stock solution.
3. Fill to final volume of 1 L with dH<sub>2</sub>O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of HEPES free acid ([HEPES, Free Acid, GoldBio Catalog # H-400](#) [CAS 7365-45-9, mw. = 238.30]) and HEPES sodium salt can be mixed to attain a pH of ~ 7.5. The pH can be adjusted by increasing the molar ratio of HEPES free acid (more acidic) or HEPES sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 1M HEPES, use the table below to estimate the required volume of acid for a given pH:

Starting pH: 10.56  
Adjust pH with: conc. HCl

pH	6.8	6.9	7	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8	8.1	8.2	8.3
mL	75	73	71	68	65	62	59	55	50	46	43	37	32	28	24	21

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

HEPES pKa at 25°: 7.50  
HEPES pH range: 6.8 – 8.2  
d(pKa)/dT value: -0.014