

Stock Solution



TD-S Revision 2.0

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1M HEPES Buffer - 1 L

Instructions

1. Dissolve 238.30 g of HEPES ([HEPES, Free Acid, GoldBio Catalog # H-400](#) [CAS 7365-45-9, mw. = 238.30]) in 750 mL of dH₂O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH₂O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of HEPES free acid and HEPES sodium salt ([HEPES, Sodium Salt, GoldBio Catalog # H-401](#) [CAS 75277-39-3, mw. = 260.29]) 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 base for a given pH:

Starting pH: 5.63
Adjust pH with: 10N NaOH

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
mL	6	8	10	14	17	20	24	28	33	38	44	49	55	61	67

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