



**Title:** *Buffers and Stock Solutions: Hanks' Balanced Salt Solution (HBSS)*

No: RTLP-GL-BSS-14

Location:  
*Old CCRC Tripp Lab*

Approval Date:  
10 September 2004

Supersedes Date:

**Materials:**

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- |   |  |                     |                  |
|---|--|---------------------|------------------|
| •Lab coat   | •KCL   | •Stir plate         | •Pipettes        |
| •Gloves   | •Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O | •Stir bar           | •Pipetteman      |
| •Filter unit: cellulose acetate membrane, 0.2µm pore size | •KH <sub>2</sub> PO <sub>4</sub>                     | •pH Meter           | •Pipette Aid     |
|   | •NaHCO <sub>3</sub>                                  | •1 M HCL            | •Pipetteman tips |
|   | •CaCl <sub>2</sub>                                   | •1 M NaOH           |                  |
|   | •MgCl <sub>2</sub> ·6H <sub>2</sub> O                | •dH <sub>2</sub> O  |                  |
|   | •MgSO <sub>4</sub> ·7H <sub>2</sub> O                | •Analytical balance |                  |
|   | •NaCl  | •1L erlymeyer flask |                  |
|   | •D-glucose   |                     |                  |
|   | •phenol red  |                     |                  |

**Procedure:**

1. Weigh out and combine the following:
  - 0.40 g KCl (5.4 mM final)
  - 0.09 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O (0.3 mM final)
  - 0.06 g KH<sub>2</sub>PO<sub>4</sub> (0.4 mM final)
  - 0.35 g NaHCO<sub>3</sub> (4.2 mM final)
  - 0.14 g CaCl<sub>2</sub> (1.3 mM final)
  - 0.10 g MgCl<sub>2</sub>·6H<sub>2</sub>O (0.5 mM final)
  - 0.10 g MgSO<sub>4</sub>·7H<sub>2</sub>O (0.6 mM final)
  - 8.0 g NaCl (137 mM final)
  - 1.0 g D-glucose (5.6 mM final)
  - 0.2 g phenol red (0.02%; optional)
2. Bring this mixture up to 1L with dH<sub>2</sub>O and adjust to pH 7.4 with 1M HCL or 1 M NaOH.
3. Filter-sterilize and store up to 1 month at 4°C.

*NOTE: Alternatively, HBSS can be purchased from Biofluids or Bio-Whittaker.*

*HBSS may be made or purchased without  $Ca^{2+}$  and  $Mg^{2+}$  (CMF-HBSS). These components are optional and usually have no effect on an experiment; in a few cases, however, their presence may be detrimental. Consult individual protocols to see if the presence or absence of these components is recommended.*

*Bottles should be kept tightly closed to prevent  $CO_2$  loss and subsequent alkalization.*

Author	Management Approval/Date	Effective Date