# Text S1: Recipe for variant-Hypho medium

### Concentrations in Final Medium

| Chemical | Concentration | Purpose |
|---|---|---|
| $K_2HPO_4$ | 14.5 mM | Buffer/Nutrient |
| $NaH_2PO_4$ | 18.8 mM | Buffer/Nutrient |
| $(NH_4)_2SO_4$ | 3.8 mM | Nutrient |
| $MgSO_4$ | 0.8 mM | Nutrient |

### General Comments

This medium uses phosphates as the buffer and the pH of the final media is determined by the relative concentration of the monobasic and dibasic phosphate components. A media equivalent to this one can also be made by switching the cation used in the phosphates as long as the relative concentration stays the same (such that $K_2HPO_4 \rightarrow Na_2HPO_4$ and $NaH_2PO_4 \rightarrow KH_2PO_4$). This simple recipe does not include calcium or trace metals. Historically, 1000X of a modified Vishniac trace metal mix has been added, as shown below.

### Preparation

This media can be prepared by combining two stock solutions.

**Recipe (for 1 L):** 100 mL 10X P-solution
100 mL 10X S-solution
800 mL deionized $H_2O$

**P-solution (10x):** $K_2HPO_4$ 25.3 g (or 33.1 g $K_2HPO_4 \bullet 3\ H_2O$)
$NaH_2PO_4$ 22.5 g (or 25.9 g $NaH_2PO_4 \bullet H_2O$)
in 1 L of deionized $H_2O$

**S-solution (10x):** $(NH_4)_2SO_4$ 5 g
$MgSO_4 \bullet 7\ H_2O$ 2 g (or 0.98 g $MgSO_4$)
in 1 L of deionized $H_2O$

**Vishniac Trace Elements (1000X):**

Add in the following order, adjusting the pH to 5.0 with each addition.

| | |
|---|---|
| $dH_2O$ | 500 mL |
| EDTA | 5 g (or 6.37 g of EDTA •2 $H_2O$) |
| $ZnSO_4$ •7 $H_2O$ | 2.2 g |
| $CaCl_2$ •2 $H_2O$ | 0.733 g |
| $MnCl_2$ •4$H_2O$ | 0.506 g |
| $FeSO_4$ •7 $H_2O$ | 0.499 g |
| $(NH_4)MO_7O_{24}$ •4 $H_2O$ | 0.110 g |
| $CuSO_4$ •5 $H_2O$ | 0.157 g |
| $CoCl_2$ •6 $H_2O$ | 0.161 g |

Note: This recipe is based on a trace metal formula given in the fourth footnote of a review paper by Vishniac and Saunter (Vishniac W & Santer M (1957) The Thiobacilli. *Bacteriological Reviews* 21:195.). However, the original formula had 50 fold higher concentrations and used a pH of 6.0.