

### **ATCC Medium: 2700 50X Heterotrophic Basal Salts Solution**

Growth medium for BAA-BAA-1645 (Y005), BAA-1647 (T23) and BAA-1648 (BRGM2).

#### **Complete Medium**

50X heterotrophic basal salts (see below)	20.00 ml
Trace elements solution (see below)	1.00 ml
Yeast Extract	0.20 g
Distilled water	980.00 ml

**The medium should be at a final pH of 2.0 for BAA-1645 and BAA-1647**

**The medium should be at a final pH of 1.8 for BAA-1648**

*Adjust the pH of the medium to 2.0 with H<sub>2</sub>SO<sub>4</sub>.*

*Distribute 5-6 ml of medium into screw cap test tubes. When working with larger volumes, dispense 49 ml of medium into 250 ml screw cap flasks. Autoclave at 121°C for 20 minutes.*

*When the medium has cooled:*

- add 1mL ferrous sulfate (1M stock solution-see below)
- add 0.5 ml (1M glycerol stock solution-see below)

#### **Growth medium for BAA-1646 (WJB-3).**

50X heterotrophic basal salts (see below)	20.00 ml
Trace elements solution (see below)	1.00 ml
Distilled water	980.00 ml

**The medium should be at a final pH of 3.0 for BAA-1646.**

*Distribute 5-6 ml of medium into screw cap test tubes. When working with larger volumes, dispense 49 ml of medium into 250 ml screw cap flasks. Autoclave at 121°C for 20 minutes.*

*When the medium has cooled:*

- add 10 µl ferrous sulfate ( 1M stock solution-see below)
- add 50 µl fructose (1M stock solution-see below).

**50X Basal Salt Solution:**

Add 20.00 ml to each liter of complete medium

Na <sub>2</sub> SO <sub>4</sub> ·10H <sub>2</sub> O	7.50 g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	22.50 g
KCl	2.50 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	25.00 g
KH <sub>2</sub> PO <sub>4</sub>	2.50 g
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	0.70 g
Distilled water	1.0L

**Autoclave @ 121°C****Stock solutions:**

Ferrous Sulfate solution:

1M stock solution adjusted to pH 1.8 with H<sub>2</sub>SO<sub>4</sub>. Filter Sterilize.

Glycerol Solution:

1M stock solution of glycerol. Autoclave @ 121°C

Fructose Solution:

1M Fructose. Filter Sterilize

**Trace elements solution (ATCC Mineral Solution (MD-TMS) may be substituted.)**

Add 1ml to each liter of complete medium

ZnSO <sub>4</sub> · 7H <sub>2</sub> O	10.00 g
CuSO <sub>4</sub> · 5H <sub>2</sub> O	1.00 g
MnSO <sub>4</sub> · 4H <sub>2</sub> O	1.00 g
CoSO <sub>4</sub> · 7H <sub>2</sub> O	1.00 g
Cr <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·15H <sub>2</sub> O	0.50 g
H <sub>3</sub> BO <sub>3</sub>	0.60 g
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	0.50 g
NiSO <sub>4</sub> · 6H <sub>2</sub> O	1.00 g
Na <sub>2</sub> SeO <sub>3</sub> ·10H <sub>2</sub> O	1.00 g
Na <sub>2</sub> WO <sub>4</sub> · 2H <sub>2</sub> O	0.10 g
NaVO <sub>3</sub>	0.10 g
Distilled Water	1.00L

**Preparation:** Adjust the pH of 800 ml of water to 2.0 using H<sub>2</sub>SO<sub>4</sub>. Add the above salts in the order listed allowing each to dissolve before adding the next. Monitor the pH keeping it at 2.0. After the addition of the Vanadate (NaVO<sub>3</sub>), increase the volume to 1 liter. Autoclave @ 121°C. Final pH 2.0

**Note: Following autoclaving -the Vanadate will require up to several days to dissolve. If you do not have Sodium Vanadate, ATCC Mineral Solution (MD-TMS) may be substituted.**