

## **ATCC Medium 958: Hemin Medium for Mycobacterium**

### **Agar Medium**

Middlebrook 7H10 agar.....	19.0 g
Glycerol.....	5.0 mL
Dubos Oleic Albumin Complex (OADC) (see below)...	100.0 mL
DI Water.....	900.0 mL
Hemin solution (see below).....	1.0 mL

Mix all components except for OADC.  
Autoclave at 121°C.  
Cool to 50-55°C and aseptically add OADC.

### **OADC**

Oleic Acid (Sigma Cat # O-1008).....	0.05 g
Albumin Fraction V Bovine (Sigma A9647).....	5.0 g
Dextrose (BD 215530).....	2.0 g
Catalase (beef) (Sigma C-9322).....	0.004 g
NaCl.....	0.85 g
DI Water.....	100.0 mL

Filter sterilize and aseptically add to base medium.

Note: Completed OADC is available from BD (237510) or can be made following the formulation above.

### **Broth Medium**

Middlebrook 7H9 Broth.....	4.7 g
Glycerol.....	2.0 mL
ADC Enrichment (see below) .....	100.0 mL
DI Water.....	900.0 mL
Hemin solution (see below).....	1.0 mL

Mix all components except for ADC.  
Autoclave at 121°C. Cool to 50-55°C.  
Aseptically add filter sterilized ADC Enrichment.

### **ADC Enrichment**

Bovine Serum Albumin, Frac V.....	5.0 g
Dextrose.....	2.0 g
Catalase (beef).....	3.0 mg
DI Water.....	100.0 mL

Filter sterilize and aseptically add to base medium.

### **Hemin Solution**

Hemin..... 0.039 g  
1N NaOH..... 1.0 mL

Mix well and add to base medium.

*To achieve concentration of hemin:*

60 micro moles of hemin

FW = 651.94g/1000,000  $\mu$ mol

60  $\mu$ mol =  $\frac{651.94 \text{ g} \times 60 \mu\text{mol}}{1000,000 \mu\text{mol}}$  = 0.039 g

### **Final Product Description**



- Medium should be translucent and brown/slightly dark green in color.
- Recommended storage temperature: 2-8°C.