

## **ATCC Medium: 243 HI Plus Medium w/ YE #3**

### **Base Medium**

|                               |          |
|-------------------------------|----------|
| Heart Infusion Broth.....     | 25.0 g   |
| Agar Noble (if required)..... | 15.0 g   |
| DI Water.....                 | 700.0 mL |

Autoclave at 121°C. Cool to 55°C and aseptically add medium supplements:

### **Medium Supplements**

|                                              |          |
|----------------------------------------------|----------|
| Horse serum (heat-inactivated).....          | 200.0 mL |
| Yeast Extract #3 (ATCC 9678, see below)..... | 100.0 mL |

\*Horse serum should be heat inactivated at 56°C for 30 minutes.

When making with agar, be sure to warm serum and yeast extract solution to 55°C before adding to basal medium.

Final pH should be **7.4**. Dispense as desired.

### **Yeast Extract #3**

|                                               |           |
|-----------------------------------------------|-----------|
| Yeast (Store bought Fleischmann's yeast)..... | 150.0 g   |
| DI Water.....                                 | 1000.0 mL |

Mix together for 10 minutes, autoclave at 121°C. Let stand overnight. Transfer yeast solution to centrifuge bottles and spin at 9,000 rpms for 50 minutes. Carefully pour supernatant into 100 mL bottles and discard the cell debris. Autoclave at 121°C and store sterile at 2-8°C.