

## **ATCC Medium 461: Castenholz TYE Medium**

### **Complete Medium**

Castenholz Salts, 2x (see below) .....	500.0 mL
1% TYE Solution (see below) .....	100.0 mL
DI Water .....	400.0 mL
Agar (if required, 1.5%) .....	15.0 g
Agar (alternative) .....	30.0 g

*Heat and mix TYE solution, DI water and agar (if required). Autoclave at 121° C.*

*Aseptically add salt solution.*

***Adjust for final pH 7.6***

*Dispense as required.*

### **Catenholz Salts, 2x**

Nitritotriacetic acid .....	0.2 g
Nitsch's trace elements (see below) .....	2.0 mL
FeCl <sub>3</sub> solution (0.03%, see below) .....	2.0 mL
CaSO <sub>4</sub> x 2H <sub>2</sub> O .....	0.12 g
MgSO <sub>4</sub> x 7H <sub>2</sub> O .....	0.2 g
NaCl .....	0.016 g
KNO <sub>3</sub> .....	0.21 g
NaNO <sub>3</sub> .....	1.4 g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.22 g
DI Water .....	1000.0 mL

***Adjust pH to 8.2.***

*Filter sterilize portion prior to use.*

*May be stored at 2-5° C for one year.*

### **Nitsch's Trace Elements**

H <sub>2</sub> SO <sub>4</sub> .....	0.5 g
MnSO <sub>4</sub> .....	2.2 g
ZnSO <sub>4</sub> .....	0.5 g
H <sub>3</sub> BO <sub>4</sub> .....	0.5 g
CUSO <sub>4</sub> .....	0.016 g
Na <sub>2</sub> MoO <sub>4</sub> .....	0.025 g
CoCl <sub>2</sub> x 6H <sub>2</sub> O .....	0.046 g
DI Water .....	1000.0 mL

*Mix and add to salt solution*

### **FeCl<sub>3</sub> Solution (0.03%)**

FeCl <sub>3</sub> .....	0.03 g
DI Water.....	100 mL

*Mix and add to salt solution.*

**1% TYE Solution**

Tryptone .....	10.0 g
Yeast Extract .....	10.0 g
DI Water .....	1000.0 mL

*Autoclave at 121° C for 15 minutes.*