

## *Aspergillus fumigatus* Media and Protoplast Transformation Recipes

### **Stock Solutions:**

#### **20X Salt Solution**

NaNO <sub>3</sub>	120 g
KCL	10.4 g
MgSO <sub>4</sub> •7H <sub>2</sub> O	10.4 g
KH <sub>2</sub> PO <sub>4</sub>	30.4 g
ddH <sub>2</sub> O to 1 Liter	

Store at Room Temperature

#### **Trace Elements Solution**

ZnSO <sub>4</sub> •7H <sub>2</sub> O	2.2 g
H <sub>3</sub> BO <sub>3</sub>	1.1 g
MnCl <sub>2</sub> •4H <sub>2</sub> O	0.5 g
FeSO <sub>4</sub> •7H <sub>2</sub> O	0.5 g
CoCl <sub>2</sub> •5H <sub>2</sub> O	0.16 g
CuSO <sub>4</sub> •5H <sub>2</sub> O	0.16 g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> •4H <sub>2</sub> O	0.11 g
Na <sub>4</sub> EDTA	5.0 g

Add solids in order to 80 ml of ddH<sub>2</sub>O dissolving each to completion before adding next. Heat the solution to boiling, cool to 60°C, adjust pH to 6.5-6.8 with KOH pellets, cool to room temperature, and adjust volume to 100 ml with ddH<sub>2</sub>O. The final solution is dark brown and will settle. Mix before use by shaking.

## **Aspergillus Tissue Culture:**

### **Glucose Minimal Medium (GMM)**

For 1 L:

20X Salt Solution	50 ml
Trace Elements	1 ml
D-Glucose (Dextrose)	10 g
Agar	15 g

#### **Supplements if using Auxotrophs**

Uracil	0.56 g
Uridine	1.26 g

pH to 6.5, dH<sub>2</sub>O to 1 L and AUTOCLAVE for 20 minutes

\*\*Can Substitute different Carbon Sources for Glucose, i.e. glycerol, or ethanol etc.

### **Liquid Glucose Minimal Medium (LGMM)**

\*This is used for general fungal growth for DNA extractions and for preparing overnight tissue prior to protoplast transformations.

Same Recipe as GMM except **NO AGAR** and add 0.5% Yeast Extract.

### **Stabilized Minimal Medium 1.5% (SMM)**

\*Used for plating transformed protoplasts

For 1 L:

20X Salt Solution	50 ml
Trace Elements	1 ml
D-Glucose (Dextrose)	10 g
Agar	15 g
Sorbitol	218,6 g (1.2M)
Yeast Extract	1 g

pH to 6.5, dH<sub>2</sub>O to 1 L

Autoclave 20 minutes

**\*\*For 0.7% Top Agar use SMM recipe with 7 g agar/L**

## **Protoplasting Media and Solutions:**

### **Osmotic Medium (OM)**

For 500 ml:

1.2 M MgSO<sub>4</sub> (147.88g of MgSO<sub>4</sub>·7H<sub>2</sub>O)

10 mM Sodium Phosphate Buffer (2.5 ml of 2M Sodium Phosphate Buffer)

2 M Sodium Phosphate Buffer Stock

For 100 ml:

Na<sub>2</sub>HPO<sub>4</sub> 9.09 g

NaH<sub>2</sub>PO<sub>4</sub> 16.34 g **OR** 18.79g of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O

pH to 6.5

Adjust pH to 5.8 with 1M Na<sub>2</sub>HPO<sub>4</sub>

**Filter Sterilize** and store at 4°C

### **Protoplast Trapping Buffer**

For 1 L:

0.6 M Sorbitol (109.3 g of Sorbitol)

0.1 M Tris-HCl, pH 7.0 (100 ml of 1 M Tris-HCl, pH 7.0 stock)

1 M Tris-HCl, pH 7.0

For 500 ml:

60.7 g Tris (in approx 400 ml water), pH to 7.0 with HCl, water to 500 ml

Aliquot in 100 ml, autoclave, store at 4°C

### **STC Buffer**

For 1L:

1.2 M Sorbitol = 218.6 g

10 mM CaCl<sub>2</sub> = 1.47 g of CaCl<sub>2</sub>·2H<sub>2</sub>O

10 mM Tris-HCl, pH 7.5 = 10 ml of 1 M Tris-HCl, pH 7.5 stock

Aliquot in 50 ml, autoclave, store at 4°C

## **Protoplasting Media and Solutions (Cont):**

### **Polyethylene Glycol (PEG) Solution**

For 100 ml:

60% PEG = 60 g of PEG 3350

50 mM  $\text{CaCl}_2$  = 0.735 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

50 mM Tris-HCl, pH 7.5 = 5 ml of 1 M Tris-HCl, pH 7.5 stock

\*final volume needs to be 100 ml, PEG takes up a lot of volume, slowly dissolved PEG into the Tris solution plus about 30 ml of water, slowly add more water as needed, but be careful not to exceed 100 ml of final volume.

Autoclave, store at room temp.