Aspergillus fumigatus Media and Protoplast Transformation Recipes

Stock Solutions:

20X Salt Solution

$NaNO_3$	120 g
KCL	10.4 g
$MgSO_4 \bullet 7H_2O$	10.4 g
KH_2PO_4	30.4 g
ddH_2O to 1 Liter	

Store at Room Temperature

Trace Elements Solution

$ZnSO_4$ $\bullet 7H_2O$	2.2 g
H_3BO_3	1.1 g
$MnCl_2 \bullet 4H_2O$	0.5 g
$FeSO_4 \bullet 7H_2O$	0.5 g
$CoCl_2 \bullet 5H_2O$	0.16 g
$CuSO_4 \bullet 5H_2O$	0.16 g
$(NH_4)6Mo_7O_{24} \bullet 4H_2O$	0.11 g
Na_4EDTA	5.0 g

Add solids in order to 80 ml of ddH_2O 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_2O. 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_2O 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_2O 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 $MgSO_4$ (147.88g of $MgSO_4$-$7H_2O$)
10 mM Sodium Phosphate Buffer (2.5 ml of 2M Sodium Phosphate Buffer)

2 M Sodium Phosphate Buffer Stock
For 100 ml:
Na_2HPO_4 9.09 g
NaH_2PO_4 16.34 g **OR** 18.79g of $NaH_2PO_4 \bullet H_2O$
pH to 6.5

Adjust pH to 5.8 with 1M Na_2HPO_4
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 $CaCl_2$ = 1.47 g of $CaCl_2 \bullet 2H_2O$
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 $CaCl_2$ = 0.735 g of $CaCl_2 \bullet 2H_2O$
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.