# Protoplast preparation for *A nidulans*

## Introduction

This procedure efficiently produces clean protoplasts that can be used for transformation or DNA extraction

## Materials

**AMM líquid**
Glucose 10 g
Ammonium tartrate 0,92 g
KCl 0,52 g
MgSO4-7 H2O 0,52 g
KH2PO4 1,52 g
Vitamines 1 X
Uracil 0,6 g
Uridine 0,6 g
Trace Element* 1 ml
H2O 1 litro
Adjust pH to 6,8 with NaOH
Autoclave
*Same used for PDA medium

**Washing buffer (1 l)**
0,6 M MgSO4 - 7 H20 148 g
Mw MgSO4 - 7H2O = 246,68
Mw MgSO4 - H2O = 120,5

**TP buffer 0,1 l**
1,2 M MgSO4 - 7H20 29,6 g
100 mM sodium phosphate pH 5,8-6,0
Filter sterileize

**ST100 buffer 0,1 l**
0,6 M sorbitol 10,93 g
100 mM Tris-ClH pH 7,0
Mw Sorbitol = 182,2
Mw Tris = 121,1

**ST10 buffer 0,25 l**
1 M sorbitol 45,55 g
10 mM Tris-ClH pH 7,5

**MSC buffer 0,1 l**
10 mM MOPS pH 6,5
1 M sorbitol 18,22 g
10 mM CaCl2 0,15 g
Mw MOPS = 209,3
Mw CaCl2 = 147,02

**PEG solution**
60% (w/v)PEG 4000 or 6000 dissolved in MSC

## Procedure

1. Obtain fresh conidia from PDA plates.
2. Inoculate 100ml of AMM minimal medium in 1 litre flask with a final concentration of 5x $10^7$ /ml. Allow to grow for 5h with shaking at $37^0$C to induce germination. Check by microscopy.
3. Centrifuge at 4000 rpm to pellet germinated conidia. Wash them with 200ml of washing buffer, re-centrifuge, leave pellet to air dry and weigh.
4. Resuspend mycelia (A) in TP (1ml/40mg of micelia) supplemented with 10mg/ml of lysing enzymes freshly made and filter sterilized (B).
5. Incubate for 2h at $28^0$C with gentle shaking (100rpm) until complete cell wall degradation. Microscope check protoplast formation every half hour (C). Transfer to 10 ml sterile tube.
6. Overlay protoplast suspension with an equal volume of ST100 buffer (D). Centrifuge 1500g at RT for 15 mins. Protoplasts will form a sharp

layer at the interface (E).
7. Transfer protoplast layer to a new sterile tube and dilute to 10-15ml with ST10 buffer carefully.
8. Protoplasts can be stored at $4^0$C until transformation.

NOTES

A. Resuspend mycelia thoroughy since conidia tend to form aggregates and then the solution of lysing enzymes can not degrade the cell walls properly.
B. Dissolve enzyme first in milliQ water ph 6.0 add magnesium sulphate 2x, phosphate buffer 10x and water to achieve final concentration and volume.
C. Protoplasts are seen as dark spherical cells. If add drop of 10% SDS while checking protoplasts will rupture, whilst conidia will not be affected.
D. Add buffer VERY slowly down the tube wall.
E. Protoplasts appear as a whitish dusty interphase.
F. Make dilutions to count cells in MSC, protoplast will burst if dilutions are made in water.

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