

Maintenance of BY-2 cell cultures

Culture Medium

modified MS (after Nagato 1992)

per liter

4.3 g MS salts (Sigma M5524) (in fridge)
10 ml thiamine (0.1 g/l) + myo-inositol (10 g/l)
3.5 ml Miller's (60 g/l KH_2PO_4)
2 ml 2,4-D (100 mg/l)
30 g sucrose
pH 5.6 with KOH

autoclave and store in airtight bottle for up to a month or so

for plates: add 1.2 g phytigel per 0.5 l

Antibiotics for selection (for example)

500 $\mu\text{g/ml}$ carbenicillin (to kill bacteria, stock is 250 mg/ml in H_2O)

50 $\mu\text{g/ml}$ kanamycin (to select for transformed BY-2, stock is 50 mg/ml in H_2O)

Growth

liquid:

on orbital shaker (120 rpm) at 27°C in the dark

callus:

at 27°C in the dark

Transfer Cultures

liquid:

EVERY WEEK, 1:50 (i.e. 400 μl per 20 ml MS)

keep old cultures around for a week on RT shaker as backups

callus:

EVERY 3 WEEKS, wrap with parafilm and keep in airtight box in incubator (to prevent drying out)