

Culturing of BY-2 suspension cells

Introduction

- Use of BY-2 suspension cells to study the cell cycle including mitotic division
- Generation and culturing of BY-2 cells
- Adapted from: Graumann K and Evans DE, (2014), "Dynamics of the Plant Nuclear Envelope during Cell Division", Methods in Molecular Biology

Materials

Hormone stock solution

2,4-Dichlorophenoxyacetic acid (2,4-D), 1 mg/ml in ethanol

1. Dissolve 1 mg 2,4-D in 1 ml 70% (v/v) ethanol.
2. Store up to 6 to 8 weeks at 4°C.

Antibiotics

Antibiotics should be prepared according to manufacturers instructions and sterilised as follows

1. Aspirate the solution into a sterile syringe without a needle.
2. Attach a 0.2- μ m syringe filter and expel the solution through the filter into a sterile tube.

Medium for suspension cultures of BY-2 cells

1. Place 750 ml water in a 1-litre beaker and add 30 g AnalaR grade sucrose. Stir until dissolved.
2. Add 4.3 g Murashige and Skoog medium (without sucrose, indole acetic acid, kinetin, agar; ICN Biomedicals)
3. Add 200 ml 1 mg/ml 2,4-D.
4. Adjust pH to 5.8 with 0.1 M KOH.
5. Make up to 1 000 ml with water, mix well, and autoclave. Allow to cool and store up to 2 weeks at 4° to 8°C.

Medium for culturing BY-2 callus

1. Add agar (plant cell specific; Bacto Agar; Difco) to liquid BY-2 medium (see above) to a final concentration of 1% (w/v).
2. Autoclave and allow to cool until flask can be held for pouring.
3. Store medium until required. Pour plates fresh just before use. For selection of stable transformants, add timentin, carbenicillin and appropriate selection antibiotics.

Avoid storing plates as this will affect potency of the antibiotics. Stored plates without antibiotics tend to become more easily infected due to condensation.

Other materials for generating BY-2 cell suspension cultures

1. Petri dish containing callus of wild-type or transformed BY-2 cells grown on solid BY-2 medium in a petri dish (obtained from a lab working with BY-2 cell lines; this is the best way to store and transport the cell lines as they require constant agitation while in liquid culture).
2. Conical flasks (50 ml) containing 20 ml liquid BY-2 medium covered with aluminium foil caps and autoclaved.
3. Conical flask (100 ml) containing 50ml liquid BY-2 medium (alternatively, 250 ml conical flask containing 100 ml liquid BY-2 medium).
4. Filter-sterilized antibiotics as required for selection of transformed BY-2 cells.
5. Sterile single edged razor blades.
6. Sterile packet of aluminium foil squares for covering flasks.
7. Orbital shaking incubator at 130 rpm, 25 °C, no light.
8. Sterile wide bore pipette.
9. Laminar flow hood.

Methods

Production of BY-2 cell suspension culture

1. Work in sterile conditions in a laminar flow cabinet.
2. Cut a 2-3 mm diameter piece of wild-type or transformed BY-2 callus with a sterile razor blade without removing it from the petri dish on which it is growing. If single, isolated calli on the plate, use 1 callus. Using 1 callus will make the culture more homogenous.
3. Holding it facing into the sterile air flow, open a sterile 50 ml conical flask containing 20 ml liquid BY-2 medium. Add filter-sterilized antibiotics as required – not required for wild type cells.

4. Open a sterile packet of aluminium foil squares.
5. Transfer the cut callus to the 50 ml flask with medium.
6. Gently pipette the culturing medium up and down to break up callus. Alternatively, use sterile forceps or a sterile pipette tip to squash the callus against the glass side of the flask.
7. Seal with aluminium foil and place in an orbital incubator (130 rpm and 25°C, no light).
8. After 1 to 2 weeks the culture should be quite thick; passage by pipetting into fresh medium, using a wide-bore pipette tip. Depending on growth rate of culture transfer 0.5-2 ml of old culture to make the new culture. Passage cells into a larger culture – 50 ml medium in 100 ml conical flask. Repeat step 8 every 7 days until the culture is growing stably.
9. Suspension cultures can be further bulked up by using 100 ml medium in 250 ml conical flasks. Depending on growth rate use up to 3 ml of old culture to transfer into 100 ml new culture medium.
10. Established cultures can be used after 4 passages for downstream applications.