Hormone screening assay

Determines what concentration of your hormones will induce regeneration of plantlets.

Prepares two petri dishes per hormone concentration.

Workflow: pour 50 mL MS medium into a 50 mL tube, add hormones, pour into two petri dishes. Repeat -> Add MS medium, add next hormone concentration, pour into two petri dishes.

2L of MS medium is prepared in two or three Scott flasks, one Schott flask is used while the others stay in a 63°C water bath so they don’t polymerize yet

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | 0 mg/L NAA | 0,1 mg/L NAA | 0,5 mg/L NAA | 1 mg/L NAA | 2 mg/L NAA | 3 mg/L NAA |
| 0 mg/L BAP |  |  |  |  |  |  |
| 0,1 mg/L BAP |  |  |  |  |  |  |
| 0,5 mg/L BAP |  |  |  |  |  |  |
| 1 mg/L BAP |  |  |  |  |  |  |
| 2 mg/L BAP |  |  |  |  |  |  |
| 3 mg/L BAP |  |  |  |  |  |  |

Our stock of NAA (frozen, stored in the freezer, aliquoted into sterile 1.5 mL eppis) is 1mg/mL.

NAA - 1 mg/mL stock

50 mL tube => 50 uL stock NAA yields 1 mg/L

BAP - 1 mg/mL stock

50 mL tube => 50 uL stock BAP yields 1 mg/L

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | 50 ml | **1** | **2** | **3** | **4** | **5** | **6** |
|  | medium | 0 uL NAA | 5 uL NAA | 25 uL NAA | 50 uL NAA | 100 uL NAA | 150 uL NAA |
| **A** | 0 uL BAP | 0B/0N | 0B/5N | 0B/25N | 0B/50N | 0B/100N | 0B/150N |
| **B** | 5 uL BAP | 5B/0N | 5B/5N | 5B/25N | 5B/50N | 5B/100N | 5B/150N |
| **C** | 25 uL BAP | 25B/0N | 25B/5N | 25B/25N | 25B/50N | 25B/100N | 25B/150N |
| **D** | 50 uL BAP | 50B/0N | 50B/5N | 50B/25N | 50B/50N | 50B/100N | 50B/150N |
| **E** | 100 uL BAP | 100B/0N | 100B/5N | 100B/25N | 100B/50N | 100B/100N | 100B/150N |
| **F** | 150 uL BAP | 150B/0N | 150B/5N | 150B/25N | 150B/50N | 150B/100N | 150B/150N |

36 tubes \* 50 mL = 1,8 L

Tip (optional): Label the petri dishes A1, A2, A3, B1 … to save a lot of time for writing

Start with low concentration, go higher (residues in the re-used 50 mL tube)

One tube per row (here 6 rows)

Duration: 2.5 hours to pour all plates

Recipe for MS Medium:

Meristemmedium (for a 500 ml Schottflask)

* 2,15 g Murashige & Skoog Basal Salt Medium
* 15 g Sucrose (Rübenzucker)
* 4 g Agar
* 500 ml deionized water - invert to dissolve at least sucrose and MS medium
* Use 120 µl NaOH to adjust pH to 5,6 – 5,8
* Autoclave for ca. 20 min at 120 – 125 °C, let cool to ca. 45-50 °C
* Pro tip: If busy, put into a Sous-Vide water bath at 63°C so it stays liquid for several hours

Add after cooling to 60°C: STERILE (in the laminar flow hood):

* 500 µl Gamborg’s Vitamin Solution (Sigma-Aldrich, Best.Nr. G1019-50ML)

+ Hormones for Tobacco:

* 50 µl of 1-Naphthalenessigsäure (1 mg/ml stock solution)
* 500 µl of 6-Benzacylaminopurin (1 mg/ml stock solution)
* Invert carefully until medium is homogenous.
* A petri dish is roughly 20-25 mL

Root regeneration after callus has formed (Tobacco):

After the plants produce sprouts in the petri dishes after roughly 3 weeks, these sprouts (shootlings) can be put into rooting medium. Rooting medium is the same as meristem-medium above but no hormones are added, and 1.5g gelzan (aka gelrite) instead of agar. This can happen in big magenta boxes or single-use 50 mL centrifuge tubes. 1/3 of the tube is filled with the medium, so the plant has space to grow in height.

If desired, antibiotic or antifungals can be added but we don’t usually do this.