Hormone screening assay

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

Prepares two petri dishes per hormone concentration.

2L of MS medium is prepared in a big Scott flask; hormones are pipetted into 50 mL tube, 50 mL MS is added.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | 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.