



Dec 03, 2025

Plant Growth on plant medium agar plates for iron deficiency response assays

DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvrw84blmk/v1>

Elke Wieneke¹, Enya Becker¹, Petra Bauer¹

¹Institute of Botany, Heinrich Heine University, Universitätsstr. 1, 40225 Düsseldorf



Petra Bauer

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.6qpvrw84blmk/v1>

Protocol Citation: Elke Wieneke, Enya Becker, Petra Bauer 2025. Plant Growth on plant medium agar plates for iron deficiency response assays. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.6qpvrw84blmk/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: August 25, 2025



Last Modified: December 03, 2025

Protocol Integer ID: 225358

Keywords: Arabidopsis thaliana, seed sterilization, seed stratification, plant growth on agar plates, Hoagland medium, Murashige and Skoog medium, iron deficiency, nutrient solution, reproducible plant cultivation, standardized growth conditions, plant physiology, root development, controlled environment growth, iron uptake, root, plant, iron nutrition, medium agar plates for iron deficiency response, iron deficiency response, iron uptake study, nutrient research, preparation of plant growth medium, nutrient medium, defined nutrient medium, iron solubility, reliable data in plant physiology, plant growth medium, plant physiology, micronutrient, arabidopsis thaliana, cultivation protocol, reproducible cultivation protocol, plant growth on plant, plant growth, cultivation of seedling, reliable cultivation, iron, consistent seedling development, uniform germination, metabolic process, synchronous seed germination, cultivation, stratification of seed, seedling

Abstract

The model plant *Arabidopsis thaliana* is widely used to study metabolic processes under defined and reproducible conditions. For reliable cultivation, plants are grown on agar plates containing a well-defined nutrient medium and maintained under standardized environmental parameters such as light intensity, day–night rhythm, and temperature. The medium provides all essential macro- and micronutrients, supplemented with sucrose to achieve equal growth. For iron uptake studies, iron is supplied in a plant-available form (FeNaEDTA) or omitted to induce iron deficiency responses, while the pH is adjusted to slightly acidic values (5.8–6.0) to ensure iron solubility and uptake. Hoagland medium is commonly used for optimal growth, although Murashige and Skoog (MS) medium can serve as an alternative.

The main task guiding this work was to establish a cultivation protocol for *Arabidopsis thaliana* that is both reproducible and transparent, allowing physiological studies particularly under iron-sufficient and iron-deficient conditions.

The aim was to adapt existing procedures and document them systematically to provide a reliable foundation for subsequent physiological studies. The work plan includes the preparation of plant growth medium with defined composition and pH, sterilization and stratification of seeds to promote uniform germination, careful sowing of seeds on agar plates under sterile conditions, and cultivation of seedlings in growth chambers under controlled environmental parameters.

Taken together, these steps enable synchronous seed germination and consistent seedling development. Reproducible cultivation protocols are essential for ensuring comparability of experiments and for generating reliable data in plant physiology.

The perspective of this work is to make such growth protocols openly available and standardized, thereby providing a methodological basis for future studies on iron nutrition and plant nutrient research in general.

Guidelines

Exemplary Workflow for the Plant Growth on plant medium agar Plates for iron Deficiency Response Assay

- 1 Sterilize *Arabidopsis thaliana* seeds using 6% NaOCl with 0.1% TRItton X-100 for 8 minutes and rinse thoroughly with sterile water.
- 2 Stratify *Arabidopsis thaliana* seeds for 2 days at 4 °C in darkness to synchronize germination.
- 3 Lay out seeds evenly on plant medium agar plates (+Fe or –Fe) under sterile conditions.
- 4 Seal plates with Parafilm and place them vertically in a growth chamber.



- 5 Depending on the experimental setup, cultivate the seedlings
 - for **6 days** on +Fe and -Fe agar plates for short-term assays, or
 - for **7-14 days** on +Fe agar plates followed by **3 additional days** after transfer to fresh +Fe or –Fe agar plates (14 + 3 system) for extended assays.

Exemplary workflow for the growth of *Arabidopsis thaliana* seedlings on plant medium agar plates under iron-sufficient (+Fe) and iron-deficient (–Fe) conditions.

The figure illustrates the main steps of seed stratification, sterilization, sowing, and vertical growth setup, followed by cultivation under long-day conditions.

Created with [BioRender.com](https://www.biorender.com).

Materials

- *Arabidopsis thaliana* seeds
- Plant agar (e.g. Plant Agar, P1001, Duchefa Biochemie, Haarlem, The Netherlands)
- Hoagland medium (or MS medium, Murashige and Skoog)
- FeNaEDTA (iron source) (e.g. Iron(III) sodium ethylenediaminetetraacetate, ≥99%, Merck KGaA, Darmstadt, Germany)
- Macronutrient and micronutrient salts (e.g. analytical grade, Carl Roth GmbH + Co. KG, Germany)
- Ferrozine (iron chelator) (e.g. 3-(2-pyridyl)-5,6-bis(4-sulfonic acid)-1,2,4-triazine disodium salt hydrate, ≥97%, Merck KGaA, Darmstadt, Germany)
- Sucrose (e.g. D(+)-Saccharose, ≥99.5%, p.a., Carl Roth GmbH + Co. KG, Germany)
- Double-distilled water (for medium preparation)
- chlorine bleach (prepared from 6% NaOCl and 0.1% Triton X-100, both from Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
- Sterile water (for washing seeds)
- Tubes for storing sterilized seeds (1.5 ml polypropylene microcentrifuge tubes (e.g. Eppendorf type))
- Autoclave
- Sterile hood/laminar flow bench
- Centrifuge (approx. 6,000 rpm, e.g. Benchmark Mini Centrifuge, Benchmark Scientific, USA)
- Pipettes and 1-ml pipette tips
- Square Petri dishes, 120 × 120 mm (≈12 × 12 cm), for a volume ~50 ml plant agar medium per plate
- Parafilm
- Paper towels
- Racks for keeping plant growth square plates upright (home-made)
- Plant growth chamber (long-day conditions: 16 h light / 8 h dark, 21 °C, ~110 μmol m⁻² s⁻¹)
- Labeling materials (marker, tape)

Sterilization solutions

a) Chlorine bleach solution (50 ml, 6% NaOCl with 0.1% Triton X-100, for seed sterilization)

b) Water (50 ml, autoclaved)

c) 0.1% Plant Agar solution (50 ml, 0.1 g plant agar dissolved in 100 ml sterile water; autoclaved).

1/2-Strength Hoagland Agar Medium (for metal uptake studies on agar plates, 50 ml per plate): combine a) to e)

a) Four Macronutrient 100X stocks (1 l to be prepared, autoclaved):

- MgSO₄·7H₂O – 18.486 g/l → 0.75 mM (final)
- KH₂PO₄ – 6.805 g/l → 0.5 mM (final)
- KNO₃ – 12.639 g/l → 1.25 mM (final)

- $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ – 35.4 g/l → 1.5 mM (final)

Dilution: 1:100 (1 ml of each stock per 100 ml medium; final = final concentration in Hoagland medium).

b) One Micronutrient 1000X stock (100 ml to be prepared, autoclaved):

- KCl – 372.8 mg/100 ml → 50 μM (final)
- H_3BO_3 – 309.2 mg/100 ml → 50 μM (final)
- $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ – 169.0 mg/100 ml → 10 μM (final)
- $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ – 57.5 mg/100 ml → 2 μM (final)
- $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ – 37.5 mg/100 ml → 1.5 μM (final)
- $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ – 9.3 mg/100 ml → 0.075 μM (final)

Dilution: 1:1000 (100 μl of each stock per 100 ml medium; final = final concentration in Hoagland medium).

c) One Iron (Fe) stock (100 ml to be prepared, autoclaved):

- FeNaEDTA – 0.368 g/100 ml → 10 mM stock

Dilution: as required for study (final = final concentration in Hoagland medium).

- Example dilutions per 100 ml medium:
- 0
- 1 μl → 0.1 μM final
- 10 μl → 1 μM final
- 100 μl → 10 μM final
- 500 μl → 50 μM final (standard control)
- 5 ml → 500 μM final

d) Ferrozine stock (light-sensitive, sterile filtration indicated, optional for use together with 0 Fe condition, usage may cause more severe iron and copper deficiency):

- Ferrozine – 2.463 g per 100 ml → 50 mM stock
- Dilute 1:1000 = 100 μl per 100 ml medium → 50 μM final

e) Agar and sucrose additions:

- Plant agar – 1.4% (w/v) in final medium (for plates)
- Sucrose – 1% (w/v) in final medium

Safety warnings

! Seed sterilization and handling

- Sterilize seeds by incubating for max. 8 min in bleach solution (6% NaOCl, 0.1% Triton-X).
- Wash thoroughly 5× with sterile water to remove residues.

Note: ethanol sterilization is not sufficient.

Sterile work and medium handling

- Handle seeds and Hoagland agar plates only under sterile conditions (laminar flow hood) to prevent contamination.
- Do not reheat prepared Hoagland medium in a microwave (salts precipitate).
- Re-autoclaving of Hoagland agar medium is not possible, as salts will precipitate/fall out.

Safety precautions

- Always follow chemical safety guidelines when handling bleach, nutrient salts, acids/bases (KOH, HCl), and during autoclaving.
- Use appropriate PPE (lab coat, gloves, eye protection) and follow chemical waste disposal procedures.

Sowing and growth orientation

- When dispensing seeds, tap the plate only very briefly and lightly to release single seeds.
- Place racks inclined backwards so that roots grow into the agar.

Sterile filtration

- Perform sterile filtration for Fe-containing stocks (FeNaEDTA) and for Ferrozine stocks, as indicated in the stock table.

Before start

- Prepare sterilization solutions.
- Prepare 1/2-strength Hoagland medium stocks.
- Fill required seeds into 1.5 ml tubes.
- Ensure access to a sterile hood/laminar flow bench, pipettes with 1000 µl pipet tips, Parafilm, plate racks, and a growth chamber set to long-day conditions (16 h light, 21 °C, ~110 µmol m⁻² s⁻¹).
- Label all plates clearly and prepare paper towels for use inside the racks.

Seed sterilization and stratification

- 1 Sterilize seeds in 1.5 ml plastic reaction tube by incubating on a rotating wheel for 8 minutes (not more) in 1 ml chlorine bleach solution.
- 1.1 Spin down seeds in a centrifuge and remove chlorine bleach solution with a pipet. Add 1 ml sterile water, swirl carefully up and down, spin down again, remove water, and repeat the washing steps 4x.
- 1.2 Finally, after washing, replace with 1 ml 0.1% Plant Agar solution (or less than 1 ml depending on seed number). For stratification, store tubes with sterilized seeds at 4°C in the dark for 2 days to synchronize germination.

Preparation of 1/2-strength Hoagland Medium Agar plates

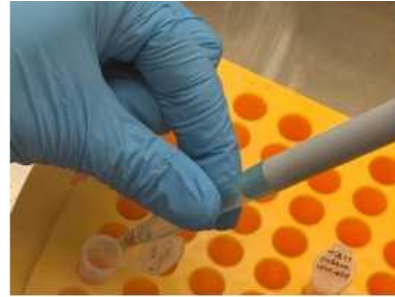
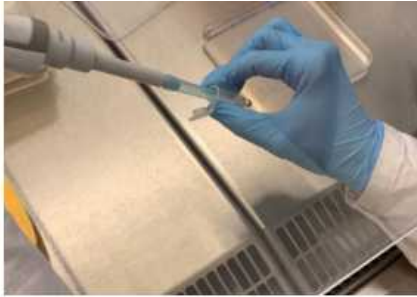
- 2 Prepare 1/2-strength Hoagland medium according to steps a) to e) in the Material section.
- 2.1 After combining a) to c), (note that d) is optional), add 1 % sucrose, and fill ca. 80% of the volume with pure water (step e).
- 2.2 Adjust the pH to 5.8 - 6.0 with KOH/HCl and fill it up to the end volume.
- 2.3 Add 1.4 % plant agar (step e).
- 2.4 Autoclave the medium. Label plates.
After autoclaving, pour plates immediately under sterile conditions (50 ml per 12 cm- 12 cm plate) or keep until then at 60°C.
Note that
Re-autoclaving or reheating solidified Hoagland agar medium in the microwave is not possible as salts precipitate. Pour plates for drying under a sterile hood for 15 min, then close lids and store plates in a plastic bag at room temperature in the dark.



Distribute seeds onto agar plates under sterile conditions

- 3 Label the plates with the name/number/treatment, your name and date.

- 3.1 With the help of a 1-ml pipet tip, take up 200 μ l of sterilized and stratified seeds. Carefully remove the pipette tip from the pipette while holding it above the tube to avoid unwanted dripping.



Keep the seed concentration in the plant agar solution low (not too dense). Regulate the quantity of seeds dispensed by adjusting the angle of inclination of the pipette tip and by avoiding pressure on the upper opening of the pipette tip.

Guide the pipette tip to the plate from below and tap the plate very briefly and gently with the pipette tip so that one seed is dispensed at a time; tapping too hard or for too long will release several seeds at once.

Approximately 15 seeds can be planted in a single row. If plants will only be grown for ~6–10 days, a second row can be placed in the middle of the plate.

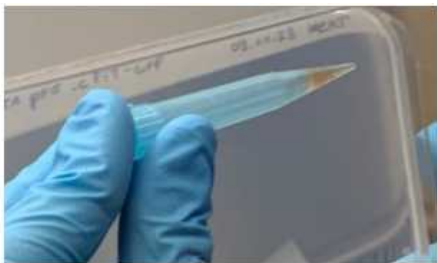


Plate handling and growth conditions

- 4 Seal plates with parafilm and place the plates into a plate-holding rack. Position the plates into the rack so the inclination points backward allowing roots to grow along the agar surface.

Place a paper towel in the middle of the rack to absorb any liquid that may leak out. An unused agar plate (also sealed with Parafilm) can be used as a shield to protect front and rear plates from excessive light and help ensure more uniform light conditions.

4.1 Place rack into the growth chamber.



4.2 Cultivate under long-day conditions (16 hours light / 8 hours dark) at 21 °C with an average light intensity of approximately $110 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Growth time:

- 6 days for short-term assays, or
- 7–14 days, followed by transfer to fresh +Fe or –Fe agar plates during an additional 3 days of growth.

Since light intensity can vary within the growth chamber due to reflections at the sides, front, or back, periodically rotate or move the racks to ensure uniform light exposure during critical experiments.



Protocol references

Hornbergs, J., Montag, K., Loschwitz, J., Mohr, I., Poschmann, G., Schnake, A., Gratz, R., Brumbarova, T., Eutebach, M., Angrand, K., Fink-Straube, C., Stühler, K., Zeier, J., Hartmann, L., Strodel, B., Ivanov, R. and Bauer, P., 2023. SEC14-GOLD protein PATELLIN2 binds IRON-REGULATED TRANSPORTER1 linking root iron uptake to vitamin E. *Plant Physiology*, 192(1), pp.504–522. <https://doi.org/10.1093/plphys/kiac563>

Lichtblau, D.M., Baby, D., Khan, M., Trofimov, K., Ari, Y., Schwarz, B. and Bauer, P., 2024. The small iron-deficiency-induced protein OLIVIA and its relation to the bHLH transcription factor POPEYE. *PLoS ONE*, 19(4), e0295732. <https://doi.org/10.1371/journal.pone.0295732>

Mohr, I., Eutebach, M., Knopf, M.C., Schommen, N., Gratz, R., Angrand, K., Genders, L., Brumbarova, T., Bauer, P. and Ivanov, R., 2024. The small ARF-like 2 GTPase TITAN5 is linked with the dynamic regulation of IRON-REGULATED TRANSPORTER 1 (IRT1). *Journal of Cell Science*, 137(23), jcs263645. <https://doi.org/10.1242/jcs.263645>

Acknowledgements

We thank all lab members that contributed to improving the protocol over the years.