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Efficient production of hydrogel-based transparent soil

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Sotta et al., 2024, under review.

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We use this protocol and it's working

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Abstract

This protocol illustrates semi-automated production of hydrogel-based "transparent soil" for plant or bacterial research. It utilizes cassette tube pump to control liquid flow and water levels to achieve stable spherification, resulting in higher yield with less labor.

Materials

-  Sodium Alginate 80-120 FUJIFILM Wako Pure Chemical Corporation Catalog #196-13325
-  Gellan Gum FUJIFILM Wako Pure Chemical Corporation Catalog #075-03075
-  Magnesium Chloride Hexahydrate FUJIFILM Wako Pure Chemical Corporation Catalog #135-00165
- Silicone tube (4 mm inner diameter, for tube A and B)
- Silicone tube (1 mm inner diameter, for tube C)
- Magnetic stirrer
- Tube holder (to fix the tube end position in a beaker)

Equipment

Cassette Tube Pump

NAME

AS ONE

BRAND

SMP-23AS

SKU

<https://www.axel-gl.com/en/asone/d/1-9419-02/?q=%20%091-9419-02>^{LINK}

Troubleshooting



Measure pumping rate

- 1 Set two tubes with different diameter to Cassette Tube Pump (ex. tube A and B, 4 mm, and tube C, 1 mm). For each tube, put one end of the tube in a beaker with water, and the other end in a empty beaker.
- 2 Turn on the pump to transfer the water to the empty beaker for 10 min.
- 3 Measure the amount of water transferred in 10 min for each tube and calculate pumping rate for each tube [mL/min].

Estimate Mg^{2+} consumption rate and adjust concentration of Mg^{2+} supply stock

5m

- 4 Prepare 1 L of 10 mM (or any desired concentration) $MgCl_2$ solution in a beaker.
- 5 Keep stirring the solution with a magnetic stirrer at 200-300 rpm.
- 6 Using the cassette pump with tube A to drop the gelling solution. Set the end of tube 1.5 cm above the water level of the $MgCl_2$ solution in a beaker.
- 7 While dropping, sample 1 mL of the $MgCl_2$ solution for every 50 mL of gelling solution.
- 8 Determine Mg concentration in each sample by ICP-AES or Titan Yellow method.
- 9 Plot the relationship between the Mg concentration and amount of the gelling solution dropped to calculate Mg consumption rate (mM/ mL gelling solution).
- 10 Determine the concentration of $MgCl_2$ supply stock based on the calculated pumping rate and Mg consumption rate.
[Mg conc. in supply stock (mM)] = [Mg consumption rate (mM/ mL gelling solution)] * [volume of $MgCl_2$ solution (L)] * [pumping rate of the dropping channel (mL/min)] / [pumping rate of the supply channel (mL/min)]

5m



Setup pumping system

- 11 Prepare 1.2 wt% polymer solution using sodium alginate and gellan gum (1:4 ratio). After autoclave cool down to room temperature.
- 12 Autoclave the beakers, tubes, magnetic stir bar, and 1 L of 10 mM MgCl_2 solution .
- 13 After surface sterilization with 70% EtOH spray, put a magnetic stirrer and Cassette Tube Pump in a laminar flow cabinet.
- 14 Pour 1 L of 10 mM MgCl_2 solution in the 1 L beaker and keep stirring on the magnetic stirrer at 200-300 rpm.

Note

If the stirring speed is too slow, the TS beads will collapse before solidification and if the stirring speed is too fast, the TS will be damaged, as well as results in formation of vortex, which affects water levels.

- 15 Setup three tubes to the cassette pump and arrange the both ends as following:
 1. Place the inlet of the dropping channel (tube A) into the bottle of gelling solution. Place the outlet on a tube holder and hold it 1.5 cm above the surface of the MgCl_2 solution and a few cm from the edge of the beaker to avoid contact between the droplets and the wall of the beaker.
 2. Place the inlet of the drain channel (tube B) near the bottom of the MgCl_2 solution, and the outlet in an empty 1 L bottle.
 3. Place the inlet of the supply channel (tube C) in MgCl_2 supply solution, and the outlet on the MgCl_2 solution in the reaction pool.

TS production and harvest

- 16 Turn on the Cassette Tube Pump to start TS production. Adjust the speed of pumping and stirrer. To fast dropping rate may cause contact among multiple drops, which could result in deformation of TS.
- 17 After dropping 400 mL of gelling solution, leave beads in the MgCl_2 solution for 15 min and then harvest them with metal sieve.

Protocol references

Reference for the production system described in this protocol.

Sotta et al., 2024, unpublished.

Original article for "transparent soil" itself. Please refer to this for usage and characteristics of transparent soil.

Ma, L., Shi, Y., Siemianowski, O., Yuan, B., Egner, T. K., Mirnezami, S.

V., ... & Cademartiri, L. (2019). Hydrogel-based transparent soils

for root phenotyping in vivo. *Proceedings of the National Academy of Sciences*, 116(22), 11063-11068.