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Protocol status: Working We use this protocol and it's working

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Bulk preparation of agarose gel

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ABSTRACT

For applications when you only need a small amount of agarose gel, it is convenient to prepare the gel in bulk ahead of time. The desired quantity of agarose can then be remelted when needed. This protocol can be modified for any agarose percentage or buffer. Fluorescent dyes for DNA visualization can be added during the remelting step.

MATERIALS

For bulk agarose prep:

- Agarose powder
- Graduated cylinder (1 L or 500 mL)
- Beaker or Erlenmeyer flask
- Lab scoops
- Weighing boats or paper
- Digital balance
- Buffer
- Microwave
- Flat tray, heat resistant

For remelting agarose gel:

- Prepared agarose gel
- Small glass beaker
- Casting stand or other mold

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SAFETY WARNINGS



- Use heat resistant mitts and eye protection when handing containers with molten agarose.
- Be careful when swirling molten agarose because it can bubble up suddenly.
- Only use glassware that is microwave safe.
- Do not pour molten agarose down the drain. Wait for it to solidify then put in the trash.

BEFORE START INSTRUCTIONS

This protocol is for 200 mL of 1% agarose, but it is scalable for any volume or percentage of agarose. For every 100 mL, use 1 g of agarose powder for 1%, 2 g for 2%, etc.

Bulk agarose gel prep

- 1 Weigh 2 g of agarose powder and add it to a beaker or Erlenmeyer flask. Use a beaker or Erlenmeyer flask that is at least 2X the volume of the agarose you are preparing.
- Add 200 mL of buffer to the beaker or flask and swirl to mix. For gel electrophoresis, use 1X TBE buffer. For Hydra culture experiments, use Hydra medium.

Note

If your application requires a precise percentage of agarose or concentration of buffer, weigh the flask before microwaving. Weigh again after microwaving and add DI water to adjust for loss of water vapor.

- Microwave at 80% power until the liquid starts to bubble. Using 80% power instead of full power will help prevent the molten agarose from boiling over. The length of time will vary depending on the type of microwave and the volume of liquid. For 200 mL, it takes about 2 minutes.
- When the liquid starts to bubble, take it out of the microwave and swirl to mix. Continue microwaving at 80% power in 30 second increments. After each 30 seconds, remove from the microwave and swirl, checking for any undissolved agarose. Continue until there are no flecks of undissolved agarose remaining.

- 5 Pour the molten agarose into a flat, shallow tray or container and allow it to solidify. Use glass or heatresistant plastic. Put the tray in the refrigerator to make it solidify faster.
- 6 When the agarose has solidified and is cool to the touch, cut it into small pieces with a lab spatula and break it apart (Figure 1). Store the pieces in a tightly sealed container in the refrigerator.



Figure 1. Agarose gel pieces.

Remelt agarose gel

7 Weigh the needed amount of agarose gel pieces into beaker. Use a beaker that is at least 2X the volume the agarose will be when melted.

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- 8 Microwave on high power for 30 seconds then swirl to mix. Continue to microwave in increments of 30 seconds, removing each time to swirl, until the agarose is completely melted.
- 9 If you are using a fluorescent dye for DNA visualization, add it now and swirl to mix it into the molten agarose.
- When the beaker is cool enough to touch with your hand, pour the molten agarose into the and allow it to completely solidify and cool to room temperature before using. The gel will appear slightly opaque when it solidifies.