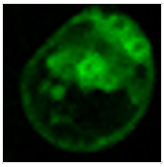

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Agarose pads for microscopy

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1 Works for me This protocol is published without a DOI.


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ABSTRACT

This protocols describe the steps required for the preparation of agarose pads for live cell fluorescent microscopy.

PROTOCOL CITATION

Joao Vitor Molino 2020. Agarose pads for microscopy. **protocols.io** https://protocols.io/view/agarose-pads-for-microscopy-bkn8kvhw

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GUIDELINES

All steps described in this protocol are intended to be conducted in a research laboratory. Follow aseptic procedures.

MATERIALS

NAME	CATALOG #	VENDOR
Frame-Seal™ in situ PCR and Hybridization Slide Chambers 15 x 15 mm 65 µl	SLF0601	

SAFETY WARNINGS

Follow the biosafety guidelines for the cell lines use during the experiment.

DISCLAIMER:

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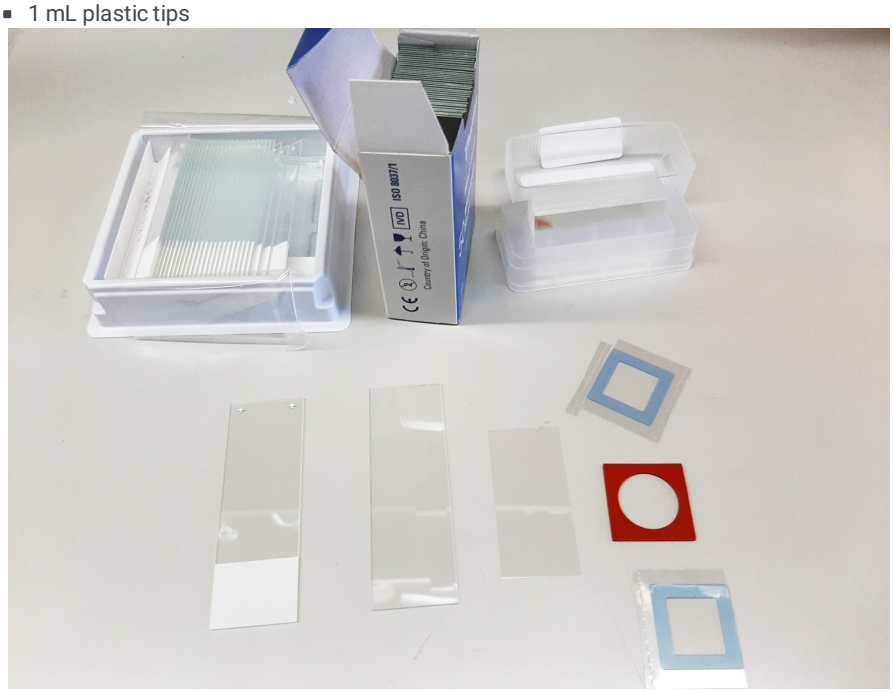
BEFORE STARTING

Separate all material needed for the protocol.
Read through the protocol.

Before start 10m

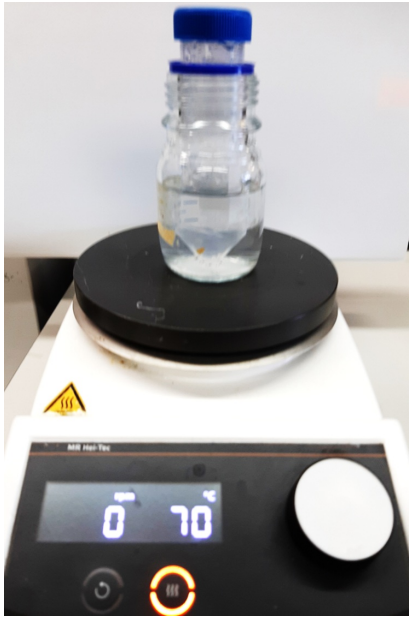
1 **Check all the necessary material:**

- Glass slide
- Cover slip
- Agarose *(Agar should work as well, but probably with less imaging quality)*
- Media or buffer
- Chamber or well forming stick (ex: Frame-Seal™ in situ PCR and Hybridization Slide Chambers, 15 x 15 mm, 65 µl)
- Tube or flask for melting agarose
- 1 mL plastic tips



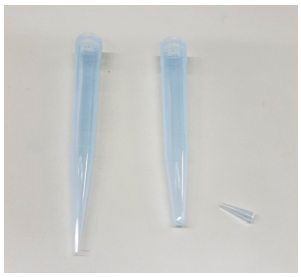
Melting agarose 5m

2 1. Melt the agarose in the desired concentration ([M] **1.5 Mass / % volume** *were tested*) using a microwave, with interval mixing. Using the desired liquid. (e.g. *Media for keeping the cells alive*)
2. Keep the melted agarose in a plate heater at **70 °C** .



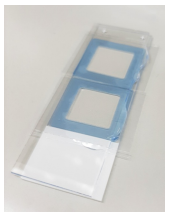
Glass slide preparation 10m

3 1. Cut a 1mL tips to increase the point diameter for easier liquid handling of the agarose.
2. Separate a glass slide to use as cover during pad preparation
3. Stick the chamber forming material to a glass slide, maintaining the plastic barrier in the exposed side *(Alternativaly you can follow the instructions at dx.doi.org/10.17504/protocols.io.kfrctm6 for a option without the chamber sticks)*
4. Add a excess of melted agarose to the well, and cover it with another glass slide for shaping the agarose pad
5. Remove the glass slide, by sliding it carefully to the side

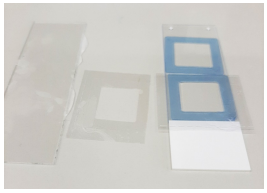


Cutted plastic tip

Melted agarose added



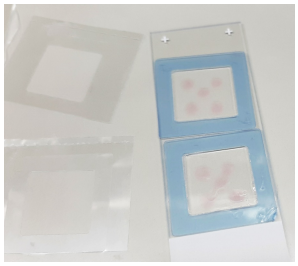
Solidifying agarose



Glass slide removed after solidification

Sample addition 10m

4 1. Add the desired amount of sample to the agarose pad. (*5-6* **1 µl** *drops were tested | Be aware of cell concentration if the goal is to see individual cells*)
2. Wait the drops to dry **00:05:00** to **00:10:00** (*Specially important for cells with motility*)
3. Carefully remove the plastic barrier exposing the adhesive face of the well forming material
4. Add a cover slip, sealing the well. (*Avoids dehydration of the agarose pad, important for long microscopy experiments)*



Samples added to agarose pad

Glass slide ready for microscopy