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Making Agarose for use in acute in vivo Electrophysiology Experiments

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Ryan Gillis[1], Mikayla Carlson[1], Severine Durand[2]

[1]Allen Institute of Neural Dynamics; [2]Allen Institute for Neural Dynamics

Allen Institute for Neural D…

Ryan Gillis
NPX

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We use this protocol and it's working

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Abstract

This protocol describes the procedure for making agarose used in acute *in vivo* Electrophysiology Experiments with Neuropixels probes. This exact methodology is crucial to the the success of these types of experiments due to the requirements that the agarose must be strong enough to provide the brain stability while also being penetrable by the delicate probes. This agarose preparation protocol is used in **Neuropixels Data Collection: Whole Hemisphere Recordings** and **Visual Cortex Recording Window Replacement for in vivo Elctrophysiology Experiments**.

Materials

Reagents

	Material	Vendor	Catalogue #	Specifications
	Certified Low-Melt Agarose	BIO-Rad	1613112	.42g
	High EEO-Agarose	Sigma-Alrich	A9793	.40g
	ACSF.V	In house	n/a	20.2mL

Equipment

	Material	Manufacturer	Part #
	125mL Erlenmeyer Flask *	Pyrex Vista	70980-125
	Metal Stir Stick *	Ibis Scientific	HS15907
	700W Microwave *	Sunbeam	
	10mL Graduated Cylinder *	Lab Depot	CY3021-10
	Hot Water Bath *	Thermo Scientific	Precision GP02
	Electronic balance *	Scout Pro	SP602
	Weigh paper *	Fisher Scientific	10347893
	2mL centrifuge tubes *	ThermoFisher Scientific	AM12425

* or equivalent

Safety warnings

Heating agarose in the microwave, while a common laboratory procedure, can result in injury if proper precautions are not taken. Agarose can become superheated and boil suddenly when removed from the microwave, resulting in splash or burn injuries to the hands, arms, and face.

Create Agarose Aliquot

1 Place a piece of weigh paper on scale

2 Make sure the scale is tared and display is showing grams.

3 Measure out .42 g of Certified Low-Melt Agarose and transfer powder to 2mL centrifuge tube.

4 On the same paper, measure .40 g of High-EEO Agarose.

5 Carefully transfer agarose powder to the same 2mL centrifuge tube and seal tightly.

> **Note**
>
> The total amount and ratio of high to low melt agarose is crucial. Take care that there is not spilling.

6 Repeat Steps 1-5 to make as many aliquots as desired.

7 Store aliquots in sealed centrifuge tubes at room temperature. They can be stored up to a year, provided they stay dry and are not exposed to any extreme conditions.

Prepare Agarose

1m 37s

8 Add the contents of one aliquot to 125-mL an autoclaved Erlenmeyer flask along with 20.2 mL ACSF.V.

> **Note**
>
> To assure effective mixing of liquid and powder components, we recommend starting by putting half the ACSF.V in the flask, followed by the aliquot components, and finishing with the second half of ACSF.V

9 Mix Agarose and ACSF.V with stainless metal stir stick, taking care not to get the mixture on edges of flask.

10 Heat in microwave for 00:01:07 on power 3 (30% of full power) then stir well to avoid bubbles in the mixture. 1m 7s

11 Heat for 00:00:15 on power 2 (20% of full power) then stir well. 15s

12 Heat for 00:00:15 on power 1 (10% of full power) then stir well. Ensure mixture is clear with no visible bubbles or granularity. 15s

13 Pour agarose mixture slowly and carefully into 10ml autoclaved graduated cylinder to avoid agitation.

Note

There will be some agarose left over in the beaker.

Safety information

Take caution working with hot agarose. Agarose can become superheated and boil suddenly when removed from the microwave, resulting in splash or burn injuries to the hands, arms, and face.

14 Store the 10ml cylinder in a water bath at 50 °C . Ensure water level in the bath is approximately even with the height of the agarose in the cylinder. Agarose can be stored in a hot water bath for at least 12 hours. After that, check for signs of drying, abnormal coloration, or excess bubbles before using.

Note

The temperature of the water bath is important for the viscosity of the agarose - if you desire your agarose to be runnier, increase the temperature by 5 °C , and do the opposite for thicker agarose.