

Materials List for

Neural Stem Cell Reactivation in Cultured *Drosophila* Brain Explants

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Corresponding Author	Citation	Citation		
Sarah E. Siegrist	Naomi Keliinui, C., Doyle, S.E., Siegrist, S.E. Neural Stem Cell Reactivation in Cultured			
ses4gr@virginia.edu	Drosophila Brain Explants. J. V	Drosophila Brain Explants. J. Vis. Exp. (183), e63189, doi:10.3791/63189 (2022).		
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Materials

Name	Company	Catalog Number	Comments
10 μL Pipette tips	Denville Sci	P2102	
1000 μL Pipette tips	Denville Sci	P2103-N	
1000 μL Pipettor	Gilson	P1000	
16% paraformaldehyde (10 x 10 mL)	Electron Microscopy Sciences	2912.60.0000	Used for Fixation of Larval Brains
20 μL Pipette	Gilson	P20	
200 μL Pipette tips	Denville Sci	1158U56	
24-well multiwell culture plates	Fisher Scientific	50-197-4477	
35 mm Petri dishes	Fisher Scientific	08-757-100A	Grape Plate Ingredients
4 °C refrigerator	Fisher Scientific		Provides an ideal temperature for>24 h incubations in antibody solution
63x Objective	Lecia		
Active dry yeast	Most supermarkets		
Agarose	Fisher Scientific	214010	Grape Plate Ingredients
Click-iT EdU Cell Proliferation Kit for Imaging, Alexa Fluor 647 dye	Thermo Fisher Scientific	C10340	to label proliferating cells
Confocal Microscope	Leica	SP8	
Coverslips 22 mm x 22 mm x 1 mm , 10 pack of 4 oz	Fisher Scientific	12-544-10	Two Coverslips are super glued to the ends of the microscope slide. This creates a space that allows for the brains to float in antifade while being imaged.
Coverslips, 22 mm x 50 mm x 1 mm	Fisher Scientific	12-545E	The coverslip is placed on two square coverslips on the microscope slide ensuring that the brain in the antifade does not move while imaging.
Dissecting microscope	Zeiss	Stemi 2000	
Ethanol 200 proof (100%), Decon Labs, 1 gallon bottle	Fisher Scientific	2701	Used to wash off the larvae before the 24 hr hold in culture medium
Fetal Bovine Serum (10%)	Sigma	F4135-100ML	Supplement for cell culture media.
Fine forceps for dissection	Fine Science Tools	11295-20	Forcepts used in disections. They work best when sharpened.



Fly Bottles for Crossing	Genessee Scientific	32-130	This bottle is used as a container that lets the flies lay eggs on the grape plate.
Glass Dissection Dish (3 well)	These are no longer available		
Glutathione	Sigma	G6013	Provides oxidative protection during cell culture.
Goat Serum	Sigma	G9023- 10ML	Blocking Agent
Grape Plates	Made in house	Made in house	Grape juice/agarose plates for collecting freshly hatched eggs
Image J	Imagej.net/fiji/downloads	Free Download: https://fiji.sc	Imaging platform that is used to count cells and Edu reactivation
Incubator	Thermo Fisher Scientific		Ensures that the temperature, humidity, and light exposure is exactly the same throughout experiment.
Insulin	Sigma	10516	Independant variable of the experiment
Laminar flow hood			For aliquoting culture media
L-Glutamine	Sigma	G7513	Provides support during cell culture
Nunc 72-well Microwell Mini Trays	Fisher Scientific	12-565-154	Immunostaining steps are performed in this tray
Parafilm	Fisher Scientific	S37440	Film used to seal plates in order to prevent evaporation
Pen-Strep	Sigma	P4458-100ml	Antibiodics used to prevent bacterial contamination of cells during culture.
Phosphate Buffer, pH7.4	Made in house	Made in house	Solvent used to wash the brains after fixing and staining steps
Pick	Fine Science Tools	10140-01	Used to pick larvae off of the grape plate
Propionic acid	Fisher Scientific	A-258	Grape Plate Ingredients
Rabbit 405	Abcam	ab175653	Antibodies used for immunostaining
Rat 555	Abcam	ab150166	Antibodies used for immunostaining
Rb Scribble	A Gift from Chris Doe		Antibodies used for immunostaining
Rt Deadpan	Abcam	ab195173	Antibodies used for immunostaining
Schneiders Culture Medium	Life Tech	21720024	Contains nutrients that help the cells grow and proliferate
SlowFade Diamond Antifade (5 x 2 mL)	Life Tech	S36963	Reagent that provides protection against fading fluorophores
Sterile Water	Autoclave Milli-Q water made in house		Needed for Solutions
Sucrose	Fisher	S2-12	Grape Plate Ingredients
Superfrost Microscope Slides	Fisher Scientific	12-544-7	
Superglue	Most supermarkets		
Tegosept	Genesee Scientific	20-259	Grape Plate Ingredients
Triton-X 100	Sigma	T9284-100ML	РВТ
Welch's 100% grape grape juice	Most supermarkets		Grape Plate Ingredients