MethodsX 4 (2017) 166–171

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Method Article

Method for organotypic tissue culture in the aged animal

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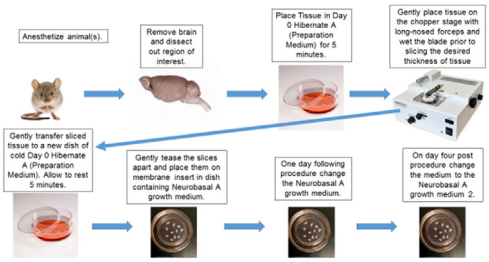
GRAPHICAL ABSTRACT



ABSTRACT

Organotypic slicing of brain tissue from young rodents has been used as a powerful model system for biomedical research [1–3]. Organotypic slicing complements cell culture and *in vivo* studies in multiple facets. This system can be useful for investigating manipulation of cellular signaling pathways without the hindrance of the blood-brain barrier while sacrificing fewer animals in the process. It also allows for preserved cellular connectivity and local intact circuitry which is a drawback of isolated cell cultures. Studies on age-related diseases have mainly used embryonic or early postnatal organotypic slice tissue. Excluding synaptic plasticity studies that are usually carried-out over a few hours and use adult mice or rats, a handful of studies performed on adult animals have had success for survival of slices [4,5]. Here we describe a method for culturing organotypic slices with high viability from hippocampus of aged mice and rabbits.

- Our method permits slices from mice as old as 16 months and rabbits as old as years of age to survive *ex vivo* up to 8 weeks [6–9]. Such a slice system may be relevant to investigating age-related brain diseases.

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http://dx.doi.org/10.1016/j.mex.2017.03.003
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ARTICLE INFO
Method name: We are describing a method for organotypic tissue culture in the aged animal in this manuscript
Keywords: Organotypic slices, Aged brain, Hippocampus
Article history: Received 20 December 2016; Accepted 31 March 2017; Available online 7 April 2017

Method details

Materials

Material	Company	Catalog Number
McIlwain Tissue Chopper	The Mickle Laboratory Engineering Co. LTD.	Model MTC/2
Teflon insert	The Mickle Laboratory Engineering Co. LTD.	
Grade 50 hardened filter paper	Whatman	1450-055
35 × 15 mm tissue culture treated dishes	Santa Cruz	Sc-200284
100 × 20 mm cell culture dishes	Greiner Bio-One	664-160
Size 2 oil paint brushes	Silver Fox	
Long-nosed forceps		
Premium Sterile Stainless Steel Scalpel Blades – #22	Havel's	FHS22
0.4 μm, 30 mm cell culture inserts	Millipore	PICMORG50
Hibernate A	Brain Bits	Hibernate A
L-Glutamine 200 mM (100×)	Gibco	25030-081
Horse Serum	Gibco	16050-122
Antibiotic/Antimycotic (100×)	Gibco	15240-062
Neurobasal-A Medium	Gibco	10888-022
2% B27 Supplement (50×)	Gibco	17504-044

Method

Preparation—Prior to animal sacrifice
Day 0 Medium Preparation
Hibernate A (preparation medium):

To a sterile 50 mL centrifuge tube add:
0.5 mM Glutamine (250 μL of stock solution)
10 mL Horse Serum
40 mL standard Hibernate A Medium

Prepare 2–3 batches if you desire extra medium and/or to change out when medium containing the slices starts to discolor (Figs. 1–3).
Neurobasal A (growth medium):

To a sterile 50 mL centrifuge tube add:
20% Horse Serum (8 mL)
400 μL standard antibiotic mixture (Antibiotic/Antimycotic)
40 mL Neurobasal A Medium

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