Rat organotypic cultures for AAV-mediated vital labeling of the extracellular matrix V.2

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ABSTRACT

In this protocol we describe the preparation and maintenance of rat organotypic cultures (**cortico-striatal**) for AAV-infection and imaging of the extracellular matrix. The AAV, in this case, induces the expression of a exportable hyaluronan-binding protein (the HA-biding domain of Neurocan) fused with GFP, that allows vital labeling (and hence live imaging) of the extracellular matrix. The organotypic cultures can be maintained for several weeks, allowing for repeated imaging and longitudinal studies.

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We use this protocol and it's working

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MATERIALS

Organotypic culture medium

50% MEM, HEPES, no glutamine **Gibco, ThermoFisher Catalog #32360026**

25% EBSS, calcium, magnesium, phenol red **Thermo Fisher Catalog #24010043**

25% Horse Serum, heat inactivated, New Zealand origin **Thermo Fisher Catalog #26050088**

1x B-27 Supplement **Gibco - Thermo Fischer Catalog #17504044**

1x GlutaMAX™ Supplement **Thermo Fisher Scientific Catalog #35050061**

36 mM Glucose Solution **Thermo Fisher Catalog #A2494001**

50 U/mL Penicillin-Streptomycin (5,000 U/mL) **Thermo Fisher Catalog #15070063**

1.25 ug/mL Fungizone (Amphotericin B) **Thermo Fisher Scientific Catalog #15290018**

Membrane Inserts

Millipore Cell Culture Inserts (0.4 um pore size, 30 mm diameter) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #PICM0RG50**

Tissue Chopper

Equipment

McIllwain Tissue Chopper NAME

Cavey Laboratory Engineering BRAND

51350 SKU

PROTOCOL MATERIALS

HBSS, no calcium, no magnesium, no phenol red **Thermo Fisher Scientific Catalog #14175095**

Step 2

Cytosine β-D-arabinofuranoside (AraC) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C1768**

Step 11

Culture preparation

1 Prepare a 6-well culture plate with Millipore 0.4 µm culture inserts placed on top of 1 mL of **organotypic medium** (see Materials) per well. Incubate it at 37 °C and 5% CO_2 to warm up.

> **Note**
>
> It is important to minimize air bubbles between the medium and the membrane insert, as this will prevent access of nutrients to the slice.

2 Prepare a p100 and p60 petri dishes with 25 mL and 5 mL, respectively, of HBSS, no calcium, no magnesium, no phenol red **Thermo Fisher Scientific Catalog #14175095** and keep them On ice .

3 Prepare the **McIllwain Tissue Chopper** (see Materials) with a fresh blade and clean holder plates.

4 Sacrifice P5-P7 rat pups by rapid decapitation and extract the brain without damaging the cortex.

5 Put the brain on a filter paper (to remove excess liquid) and cut through the midline to separate the cerebral hemispheres. Remove the midbrain, separating it from the cortex with a scalpel.

6 Place both hemispheres in the same holder plate, with the medial face downward. Hold the plate with both hemispheres perpendicular to the blade (coronal sections) and section the brain at 350 um width.

7 Pour the sliced brain carefully into the p100 petri dish with HBSS On ice . You may use a Pasteur pipette to moisten the brain and slide it more easily.

8 Separate slices under the magnification microscope with a couple of spatulas (preferentially plastic-ones, which can be custom crafted cutting the back of a Pasteur pipette). Use a blade and clean cuts to remove unwanted regions (such as hippocampus or SVZ). Transfer the slices into the p60 petri dish (you can use an inverted glass pipette with a suction rubber bulb).

Note

It is important to remove the subventricular zone (SVZ) and the meninges with the scalpel to avoid cell proliferation.

9 **Under the laminar flow hood**, transfer the selected slices (undamaged slices from the preferred rostro-caudal levels) to preheated culture inserts with medium (Step 1). Each insert can only hold two cortico-striatal slices. Remove excess dissection buffer from the top of the insert with a sterile tip.

Note

Leaving too much HBSS solution on top of the insert will eventually dilute the feeding medium.

From now on, the cultures should always be handled in sterility.

10 Incubate the culture at 37 °C and 5% CO_2.

Expected result

Typically, 4-6 cortico-striatal slices can be obtained from each hemisphere

Maintenance and AAV-infection

11 At DIV1 (24h after culture preparation), replace the feeding medium with 1 mL of the same medium but adding antimitotic

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4,4 um per well.

Expected result

AraC will prevent the overproliferation of astrocytes, minimizing the formation of a glial scar on top of the slice.

12 At DIV3, replace the AraC-containing medium with fresh medium. Then add 1 uL of the virus of choice (in our case, AAV-Ncan-GFP 10^{12} vg/mL) over the slice.

Safety information

AAVs have to be manipulated in BSL1 facilities.

Note

To improve spatial precision (e.g. when infecting a selected region from the slice), AAV infection can be done with a micromanipulator and a glass pipette.

13 To maintain the culture, change medium three times a week (1 mL per well).

Expected result

Slices will flatten overtime, and become more transparent. Cortical regions will have more cell density than striatal regions.

AAV expression become visible aprox. at DIV10, with maximum expression from DIV14 onwards (it depends on AAV serotype and promoter).

Imaging

14 Imaging can be performed in two ways:

- IN-PLATE: The insert **is not removed from the plate**, and the imaging is performed through the plastic. This is usually done in *widefield* setups, with low magnification. It is not optimal in terms of resolution, but it is very useful for repeated imaging (longitudinal studies) without compromising sterility, since the plate lid is not removed during the imaging process.
- OFF-PLATE: The insert is **removed from the plate** and placed in a smaller petri-dish or similar holding chamber. Imaging is usually done from above, in upright microscopes (objective on-top), with water-based objectives. Since CO2 is usually not available, a buffer medium with HEPES is recommended. Phenol-red should be avoided as well, since it interferes with fluorescence. This type of configuration is typically used for *confocal* or *2-photon* imaging of the slices. Repeated imaging can be done but *special attention should be paid to ensure cleanliness* during the procedure (clean objective with 70% EtOH, change medium with fresh antibiotic/antimitotic immediately after imaging, keep an eye for contamination over the following days).