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Tissue handling for mice, marmoset, and rhesus macaques injected with AAV

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We use this protocol and it's working

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Abstract

This protocol outlines all procedures for handling of tissue of animal subjects treated with AAV.

Mice tissue handling (individual AAV characterization)

- 1 Raise and house mice in compliance with your local Institutional Animal Care and Use Committee (IACUC) and ensure that all protocols and procedures have been approved by the appropriate ethical and regulatory committees.
- 1.1 On the day of perfusion (e.g. 3 weeks post-injection), perfuse mouse with PBS and fix in 4% paraformaldehyde (PFA). Extract all organs and incubate in 4% PFA overnight. Transfer into PBS supplemented with 0.01% sodium azide, and store at 4°C for long-term storage.

Marmoset tissue handling (individual AAV characterization)

- 2 Raise and house marmosets in compliance with your local Institutional Animal Care and Use Committee (IACUC) and ensure that all protocols and procedures have been approved by the appropriate ethical and regulatory committees.
- 2.1 On the day of perfusion and tissue collection (31 days post-injection), anesthetize marmosets with ketamine and euthanize using Euthasol (1 mL/kg). Perfuse with 1X PBS.
- 2.2 Cut brain and all other relevant organs (liver, spinal cord, etc.) in half. Flash-freeze one half in 2-methylbutane chilled with dry ice, and stored at -80 °C. Post-fix other half of tissue in 4% PFA overnight and then store at 4 °C in PBS + 0.025% Sodium Azide.

Rhesus macaque tissue handling (AAV pool experiments)

- 3 Raise and house macaques in compliance with your local Institutional Animal Care and Use Committee (IACUC) and ensure that all protocols and procedures have been approved by the appropriate ethical and regulatory committees
- 3.1 On the day of tissue harvest, deeply anesthetize macaques and inject sodium pentobarbital in accordance with guidelines for humane euthanasia of animals at local facility (e.g. California National Primate Research Center).
- 3.2 Perfuse macaque with ice-cold RNase-free PBS. Flash freeze one hemisphere of the brain for DNA/RNA extraction. For the other hemisphere, section into 4 mm coronal locks and post-fix in 4% PFA for 48 hours. Store in 1X PBS.

Rhesus macaque tissue handling (individual AAV characterization)

- 4 Raise and house macaques in compliance with your local Institutional Animal Care and Use Committee (IACUC) and ensure that all protocols and procedures have been approved by the appropriate ethical and regulatory committees.



- 4.1 On the day of tissue harvest, deeply anesthetize macaques and inject sodium pentobarbital in accordance with guidelines for humane euthanasia of animals at local facility (e.g. California National Primate Research Center).
- 4.2 Perfuse macaque with PBS and 4% PFA. Section entire brain to 4 mm coronal blocks and post-fixed all tissue in 4% PFA for 3 days before storage in PBS.