

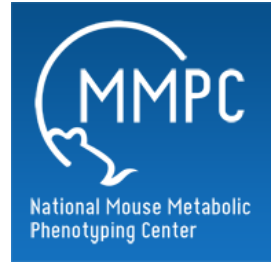


Version 1

UC Davis - Microvascular Permeability and Lipoprotein Flux V.1

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Abstract

Summary:

One of the three indices of arterial function that are compromised to a varying degree in individuals with cardiovascular disease is vascular permeability. This assay measures vascular permeability (as flux of labeled large molecular weight molecules: i.e. albumin or dextran) and lipid permeability (as flux of labeled lipid) in coronary or carotid arteries.

Materials

MATERIALS

⊗ Krebs-Henseleit Solution **Catalog #See Below**

⊗ FITC- Dextran **Merck MilliporeSigma (Sigma-Aldrich) Catalog #FD4, FD40S, or FD70**

⊗ TRITC- Dextran **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T1037 or T1162**

⊗ FITC- Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A9771**

⊗ TRITC- Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2289**

⊗ Alexa-546 label **Merck MilliporeSigma (Sigma-Aldrich) Catalog #10237**

⊗ DiI labeled Lipid **Catalog #See protocol**

⊗ pentobarbital **Cardinal Health**

⊗ DMEM **Invitrogen - Thermo Fisher Catalog #11885**

⊗ DPBS **Invitrogen - Thermo Fisher Catalog #14190**

⊗ formaldehyde **Fisher Scientific Catalog #F79**

Reagent Preparation:

Reagent 1: 10 % formaldehyde

Formaldehyde (Fisher) is diluted to 10% in DPBS (Invitrogen)

Reagent 2: Krebs-Henseleit Solution

116 mM NaCl, 5 mM KCl, 2.4 mM $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 1.2 mM MgCl_2 , 1.2 mM NH_2PO_4 , and 11mM glucose

Safety warnings

! WARNING:

Formalin is, toxic, flammable and considered a carcinogen.

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.



Before start

WARNING:

Formalin is, toxic, flammable and considered a carcinogen.

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.

- 1 Mice are anesthetized with an intraperitoneal injection with 50 mg pentobarbital/kg weight.
- 2 All treatments are administered into the left femoral vein by bolus injection. FITC-albumin (40 mg/mL) in 100 μ L:
 - a. PBS
 - b. VLDL (150 mg/dL)
 - c. VLDL (150mg/dL) + LpL (2 U/mL)
 - d. LpL (2 U/mL) in PBS
- 3 Alternatively, the mouse is then infused at with 100 uL fluorescently labeled compound alone (FLC, see above) (40 mg/mL)
- 4 Excess FLC was removed from the vasculature by infusion with DMEM media for 20 min by infusion into the left ventricle of heart and followed by infusion of 10% formaldehyde for 20 min.
- 5 The microvascular rich tissues interest are immediately removed and fixed in 10% formaldehyde for two days.
 - a. microvascular tissues = brain, heart, and mesentery ect.
 - b. macrovascular tissues= common carotid arteries or aorta
- 6 The tissue is embedded in paraffin and sectioned to 5 μ m thickness.
- 7 Tissues sections are deparaffinized, rehydrated, and imaged using fluorescent microscopy.