VERSION 1

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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

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- ☒ Krebs-Henseleit Solution **Contributed by users Catalog #See Below**
- ☒ FITC- Dextran **Sigma Aldrich Catalog #FD4, FD40S, or FD70**
- ☒ TRITC- Dextran **Sigma Aldrich Catalog #T1037 or T1162**
- ☒ FITC- Albumin **Sigma Aldrich Catalog #A9771**
- ☒ TRITC- Albumin **Sigma Aldrich Catalog #A2289**
- ☒ Alexa-546 label **Sigma Aldrich Catalog #10237**
- ☒ DiI labeled Lipid **Contributed by users 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 CaCl₂*H₂O, 1.2 mM MgCl₂, 1.2 mM NH₂PO₄, 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 INSTRUCTIONS

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- 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 μm thickness.
- 7 Tissues sections are deparaffinized, rehydrated, and imaged using fluorescent microscopy.