



Microvascular Permeability

Version: 1
 Edited by: Dr. Rutledge, UC Davis

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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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Krebs-Henseleit Solution	See Below	
FITC- Dextran	Sigma	FD4, FD40S, or FD70
TRITC- Dextran	Sigma	T1037 or T1162
FITC- Albumin	Sigma	A9771
TRITC- Albumin	Sigma	A2289
Alexa-546 label	Sigma	10237
DiL labeled Lipid	See protocol	
pentobarbital	Cardinal Health	
DMEM	Invitrogen	11885
DPBS	Invitrogen	14190
formaldehyde	Fisher	F79

Protocol:

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 μ L 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.

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