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Working

UC Davis - Microvascular Permeability and Lipoprotien Flux

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Mouse Metabolic Phenotyping Centers
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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.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=108&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Krebs-Henseleit Solution	See Below	
FITC- Dextran	FD4, FD40S, or FD70	Sigma Aldrich
TRITC- Dextran	T1037 or T1162	Sigma Aldrich
FITC- Albumin	A9771	Sigma Aldrich
TRITC- Albumin	A2289	Sigma Aldrich
Alexa-546 label	10237	Sigma Aldrich
DiL labeled Lipid	See protocol	
pentobarbital		Cardinal Health
DMEM	11885	Invitrogen - Thermo Fisher
DPBS	14190	Invitrogen - Thermo Fisher
formaldehyde	F79	Fisher Scientific

MATERIALS TEXT

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 $CaCl_2*H_2O$, 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 STARTING

WARNING:
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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 µL:
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.