U Michigan - Retinal Vascular Permeability

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ABSTRACT

Summary:

The vascular permeability is quantified by measuring albumin leakage form blood vessels into the retina. Fluorescent dye (FITC-BSA) is used to measure the breakdown in blood-retinal barrier to be detected when increased vessel leakage is extravasated into the interstitial space.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=309&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Ketamine	0409-2051-05	Hospira(Pfizer)		
Xylazine	510004	VetOne		
Heparinized micro-cuvette	Microvette 300 LH 20.1309.100	Sarstedt		
0.3 c.c. insulin syringe (31-gauge x 5/16")	328440	BD Biosciences		
1 c.c. syringe with ½" 27-gauge needle	309623	BD Biosciences		
FITC-BSA (albumin-fluorescein isothiocyanate conjugate bovine)	A9771	Sigma-aldrich		
EZ Clip wound closure	EZC KIT	Braintree Scientific		
Blunt end needle (22-gauge x ½")	Kahnetics KDS2212P	Weller		
Saline	2B1324X	Baxter		
Triton-PBS (1%)	X100	Sigma Aldrich		
Microcentrifuge tubes	C2170	Denville Scientific Inc.		
384-well microplate	Bio-One 781096	greiner bio-one		

MATERIALS TEXT

Reagent Preparation:

Reagent 1:
Ketamine/xylazine (90 and 10 mg/ml)
Procedure: Add 0.5 ml xylazine (100 mg/ml) to 4.5 ml ketamine (100 mg/ml)

Reagent 2:
FITC-BSA (100 mg/ml)
Procedure: Dissolve 1 g FITC-BSA in 10 ml sterile PBS, aliquots stored at -80°C and warmed to 37°C before use

Reagent 3:
Triton-PBS (1%)
Procedure: Dissolve 1 ml Triton X-100 in 100 ml PBS

Note:

Hospira, RRID:SCR_003985
BD Biosciences, RRID:SCR_013311
Baxter RRID:SCR_003974
Sigma-Aldrich, RRID:SCR_008988

1 Weigh animal and record body weight for anesthetic and dye injections

2 Anesthetize animal with ketamine/xylazine mixture

3 Make an incision on skin inside of the hind leg and carefully tear away the membranes to isolate the femoral vein

4 Inject FITC-BSA into the femoral vein at 2 µl/g body weight (equal to 200 mg/kg body weight) using a 31-gauge 0.3 c.c. insulin syringe (vortex FITC-BSA before use)

5 Apply pressure on the injection site with a sterile gauze or cotton swab to stop bleeding

6 Staple the incision site and allow FITC-BSA to circulate for 2 hours

7 Anesthetize the animal again with ketamine/xylazine

8 Open the abdomen and draw 0.3 ml blood from the vena cava with a 27-gauge 1 c.c. syringe

9 Remove needle and expel blood into a heparinized micro-cuvette

10 Mix blood sample by gently reversing the tube several times and keep on ice

11 Open the chest cavity, cannulate the heart with a blunt end 22-gauge needle into the left ventricle and incise right atrium to release pressure

12 Perfuse with saline (warmed to 37°C) at 20 ml/min via the left ventricle for 2 minutes

13 Harvest the retina and place in a pre-weighed microcentrifuge tube. Rinse the harvest tools between samples to avoid cross contamination

14 Centrifuge the blood sample at 2,000 x g at 20°C for 15 minutes to separate plasma

15 Transfer the plasma to a new microcentrifuge tube and store at -80°C

16 Dry the retina samples with a Speed-Vac overnight

17 Weigh the microcentrifuge tube containing the dry retina to obtain the dry retina weight

18 Add 100 µl of 1% Triton-PBS to each retina and shake overnight to extract FITC-BSA

19 Vortex briefly, centrifuge the microcentrifuge tubes at 17,000 x g for 30 minutes and transfer supernatant to a new microcentrifuge tube

20 Dilute the plasma samples with 1% Triton-PBS

21 Dilute the stock FITC-BSA (100 mg/ml) with 1% Triton-PBS and make serial dilutions to obtain the FITC-BSA standards

22 Measure the standards (0.156 to 10 µg/ml), retina extract and diluted (1:900) plasma samples (20 µl/well, triplicate) in a 384-well black/clear bottom plate with a fluorescent plate reader (excitation 488 nm, emission 520 nm)

23 Calculate the FITC-BSA concentration of each sample with the standard curve

$$\text{Permeability (µl/g/h)} = \frac{\text{(Retina FITC-BSA (µg) – Autofluorescence) / Retina Weight (g)}}{\text{Plasma FITC-BSA Concentration (µg/µl) x Circulation Time (h)}}$$

NOTE: *The auto-fluorescence background of the retina from animal without FITC-BSA injection should be subtracted for the permeability calculation.Additional tip that improves efficiency.*