Retinal Vascular Permeability

Version: 1
Replaced by version: N/A
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(note that the following list should be linked to the appropriate location.)

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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Ketamine	Hospira	0409-2051-05
Xylazine	VetOne	510004
Heparinized micro-cuvette	Sarstedt	Microvette 300 LH 20.1309.100
0.3 c.c. insulin syringe (31-gauge x 5/16”)	Becton Dickinson	328440
1 c.c. syringe with ½” 27-gauge needle	Becton Dickinson	309623
FITC-BSA (albumin-fluorescein isothiocyanate conjugate, bovine)	Sigma	A9771
EZ Clip wound closure	Braintree Scientific	EZC KIT
Blunt end needle (22-gauge x ½”)	Weller	Kahnetics KDS2212P
Saline	Baxter	2B1324X
Triton-PBS (1%)	Sigma	X100
Microcentrifuge tubes	Denville	C2170
384-well microplate	Greiner	Bio-One 781096

Protocol:

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.

Reagent Preparation:

Reagent 1
Reagent 2
Reagent 3

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