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We use this protocol and it's working

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U Michigan - Retinal Vascular Permeability

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ABSTRACT

Summary:

The vascular permeability is quantified by measuring albumin leakage from 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.

Keywords: Fluorescent dye (FITC-BSA), vascular permeability, retina

MATERIALS

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☒ Ketamine **Hospira(Pfizer) Catalog #0409-2051-05**

☒ Xylazine **VetOne Catalog #510004**

☒ Heparinized micro-cuvette **Sarstedt Catalog #Microvette 300 LH 20.1309.100**

☒ 0.3 c.c. insulin syringe (31-gauge x 5/16") **BD Biosciences Catalog #328440**

☒ 1 c.c. syringe with ½" 27-gauge needle **BD Biosciences Catalog #309623**

☒ FITC-BSA (albumin-fluorescein isothiocyanate conjugate bovine) **Sigma-aldrich Catalog #A9771**

☒ EZ Clip wound closure **Braintree Scientific Catalog #EZC KIT**

☒ Blunt end needle (22-gauge x ½") **Weller Catalog #Kahnetics KDS2212P**

☒ Saline **Baxter Catalog #2B1324X**

☒ Triton-PBS (1%) **Sigma Aldrich Catalog #X100**

☒ Microcentrifuge tubes **Denville Scientific Inc. Catalog #C2170**

☒ 384-well microplate **greiner bio-one Catalog #Bio-One 781096**

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 μ l/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

$$\text{Permeability } (\mu\text{l/g/h}) = \frac{(\text{Retina FITC-BSA } (\mu\text{g}) - \text{Autofluorescence}) / \text{Retina Weight (g)}}{\text{Plasma FITC-BSA Concentration } (\mu\text{g}/\mu\text{l}) \times \text{Circulation Time (h)}}$$

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.