

## Measuring Blood-brain-barrier Permeability Using Evans Blue in Mice

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**[Abstract]** The blood–brain barrier (BBB) is a highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the central nervous system. The blood–brain barrier allows the passage of water, some gases, and lipid soluble molecules by passive diffusion, as well as the selective transport of molecules such as glucose and amino acids that are crucial to neural function. This protocol provides a full, detailed method for measuring blood-brain-barrier permeability of mice with Evans blue (EB), that leakage was used to assess blood-brain barrier (BBB) permeability (sample: Anterior Cingulate Cortex, ACC).

### **Materials and Reagents**

1. Adult male Kunming mice (the Experimental Animal Center of Xuzhou Medical College, 20-22 g, 5-7 weeks old)
2. Evans blue (EB) (Sigma-Aldrich, catalog number: E2129-10G)
3. Trichloroacetic acid (Sigma-Aldrich, catalog number: T6399-100G)
4. Chloral hydrate (Sigma-Aldrich, catalog number: 15307-500G-R)
5. Paraformaldehyde (Sigma-Aldrich, catalog number: 158127-500G)
6. NaCl (Sigma-Aldrich, catalog number: S7653-1KG)
7. 10% chloral hydrate (see Recipes)
8. 4% paraformaldehyde-freshly-prepared (see Recipes)
9. 0.9% NaCl (see Recipes)
10. 100% trichloroacetic acid solution (see Recipes)

### **Equipment**

1. Spectrophotometer (Thermo Fisher Scientific, catalog number: 1510)
2. 96-well plate (Corning Incorporated, catalog number: 3599)
3. 1 and 20 ml syringe (local drugstore)
4. Infusion apparatus (local drugstore)

## **Procedure**

1. Mice were anesthetized with 10% chloral hydrate (0.3 ml/100 g, i. p.).
2. Injected Evans Blue (EB) (2%, 2 ml/kg) from caudal vein 0.5 h before perfusion. EB leakage was used to assess blood-brain barrier (BBB) permeability.
3. Mice were transcardially perfused with 0.9% NaCl until the perfusate from the right atrium ran clear.
4. Mice were directly beheaded, stripping brain tissue on the ice, taking the region before bregma 2.34 mm, after bregma 0.22 mm and aside the middle line 0.6 mm of the cerebral cortex on a cooled culture dish lined before experiment with a knife, which was we focus on the ACC area of the brain. The obtained ACC area was placed in the Eppendorf container cooled on the ice.
5. Each sample was weighed and then homogenized in 0.75 ml of PBS and 0.25 ml of 100% TCA solution, which could precipitate macromolecular compounds, including the nucleoprotein, using electronic homogeniser.
6. Samples were cooled overnight at 4 °C, and then centrifuged for 30 min at 1,000 x g at 4 °C.
7. The EB in the supernatants of each sample were subsequently measured at 620 nm using a 96-well plate reader, 100 µl of each sample was measured. All measurements were within the range of detection established by the standard curve.
8. The dye concentration was calculated as the ratio of absorbance relative to the amount of tissue.

## **Recipes**

1. 10% chloral hydrate  
10 g chloral hydrate powder + 100 ml double distilled water, mixed to dissolve completely
2. 4% paraformaldehyde-freshly-prepared  
20 g paraformaldehyde powder + 500 ml PB incubated at 65 °C, stirred several times to dissolve completely  
Cooled at room temperature and then added double distilled water to 500 ml for evaporated water during the course of dissolution
3. 0.9% NaCl  
9 g NaCl powder + 1,000 ml double distilled water, mixed to dissolve completely
4. 100% trichloroacetic acid solution  
500 g trichloroacetic acid solution powder + 227 ml double distilled water, mixed to dissolve completely  
Stored at 4 °C and protected from light

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