

## **Euglobulin Clot Lysis Assay for Mouse Plasma (Ze Zheng, May 2019)**

### **Required reagents:**

- **0.017% Acetic acid**  
Add 8.5uL pure acetic acid to 50mL water, freshly.
- **Sodium borate/NaCl solution, pH 9.0**  
Add 0.198 g sodium borate, and 1.8 g NaCl, to 190 mL water, adjust pH to 9.0, and bring final volume to 200 mL.
- **CaCl<sub>2</sub> (25 mM)**  
Add 0.139 g CaCl<sub>2</sub> to 50mL water.
- **Sodium Citrate**  
3.8%, weight-to-volume ratio
- **Syringe**  
For mouse cardiac puncture. I used 26G needles.

### **Procedure:**

#### **Step 1 – Plasma Collection**

1. Prepare syringe with 50uL of stock sodium citrate.
2. Collect blood from cardiac puncture. Stop drawing blood when total volume reaches. 500uL, so the blood-to-sodium citrate ratio is 9:1.
3. Centrifuge at room temperature (24C) for 15 minutes, 2300g.
4. Carefully collect plasma from the supernatant fraction. Only use fresh plasma.

#### **Step 2 – Euglobulin fraction preparation**

5. 50uL citrated plasma mixed with 900uL 0.017% acetic acid, place on ice for 20min.
6. Centrifuge 2000g for 15 min, 4C.
7. Discard supernatant. Resuspend pellet (euglobulin fraction) in 55uL sodium borate/NaCl (pH9.0).

#### **Step 3 – Euglobulin clot lysis time (ECLT)**

8. Pipette samples to flat bottom 96-well microtiter assay plates
9. Add 50uL 25mM CaCl<sub>2</sub> to each well
10. Read 405nm absorbance every 10 min for 16 hours, shake 3 sec before each read, room temp (set temp to 24C, otherwise the temp will keep go up).
11. Clot lysis time (CLT) was calculated as the time to achieve 50% of clot lysis (half-lysis time).

### **References (1-4)**

1. Z. Zheng *et al.*, An ATF6-tPA pathway in hepatocytes contributes to systemic fibrinolysis and is repressed by DACH1. *Blood* **133**, 743-753 (2019).
2. O. A. Marcos-Contreras *et al.*, Hyperfibrinolysis increases blood-brain barrier permeability by a plasmin- and bradykinin-dependent mechanism. *Blood* **128**, 2423-2434 (2016).
3. N. Ohkura *et al.*, Circadian clock molecules CLOCK and CRYs modulate fibrinolytic activity by regulating the PAI-1 gene expression. *J Thromb Haemost* **4**, 2478-2485 (2006).
4. J. Orbe *et al.*, Matrix metalloproteinase-10 effectively reduces infarct size in experimental stroke by enhancing fibrinolysis via a thrombin-activatable fibrinolysis inhibitor-mediated mechanism. *Circulation* **124**, 2909-2919 (2011).