ACarPA-FCS and ACarPA-Fib Assays

Prepare Carbamylated Fetal Calf Serum (FCS)

1. To 25 mL FCS add 2.02 g Potassium Cyanate (KCN mol. Wt 81.1 g/mole) to give 1M KCN in FCS.
2. Stir gently (set stir plate on low) until KCN goes into solution
3. Incubate 24 hr at 37 C.
4. Pre-wet dialysis tubing (7000 MW cutoff) -2X 15min soaks in barnstead water
5. Tie off one end of dialysis tubing using two knots before adding FCS. Tie off the other end with two knots.
6. Place the tubing in 2 L of barnstead water with stir bar.
7. Incubate at least 2 hr at 4 C (cold room) with constant gentle stirring.
8. Discard water and replace with 2 L of clean barnstead water. Repeat 2 more times. Note: any of the three dialyses may be left overnight at 4 C if needed.
9. Cut one end of the tubing and carefully transfer the carbamylated FCS to a 50 mL conical tube. Store at 4 C.

Prepare Carbamylated Fibrinogen

1. Purchase highly purified human fibrinogen from Hyphen BioMed (Distributed by Aniara) Cat # PP001B
2. Add 10 mL barnstead water to 100 mg of fibrinogen.
3. Prepare 1M KCN (8.1 g in 100 mL barnstead water)
4. Add 5 mL of 1M KCN to 50 mg of fibrinogen at 10 mg/mL
5. Incubate at 4 C for 3 days.
6. Dialyze against barnstead water as described for FCS.(3X 2L water for 2 hrs)
7. Carefully transfer carbamylated fibrinogen to 15 mL conical tube.
8. Aliquot 250 uL of both carbamylated and non-carbamylated fibrinogen into 1.5 mL tubes and store at -80 C.

Shi et al. www.pnas.org/cgi/content/short/1114465108

Prepare FCS ELISA Plates

1. Dilute 0.1 mL FCS and carbamylated FCS 1:100 in 9.9 mL PBS. (enough for one 96-well plate of each)
2. Add 100 uL of FCS to each well of a 96-well plate.
3. Add 100 uL of carbamylated FCS to each well of a separate 96-well plate.
4. Seal plates and incubate overnight at room temp.
5. Wash plates 3x with PBS/0.05% Tween-20
6. Add 300 uL blocking buffer-PBS/10% FCS/0.05% Tween-20
7. Seal plate-incubate overnight at room temp. Move plate to 4 C until needed.

Prepare Fibrinogen ELISA plates

1. Thaw one aliquot each of fibrinogen and carbamylated fibrinogen.
2. Dilute each to 100 ug/mL in PBS.

3. Add 100 ul of 100 ug/mL fibrinogen to each well to 96-well plate
4. Add 100 ul of 100 ug/mL carbamylated fibrinogen to each well of 96-well plate.
5. Seal plates and incubate at room temp overnight.
6. Wash plates 3x with PBS/0.05% Tween-20.
7. Add 300 uL blocking buffer-PBS/10% FCS/0.05% Tween-20
8. Seal plate-incubate overnight at room temp. Move plate to 4 C until needed.

ELISA-Same protocol for FCS and fibrinogen

1. Set up ELISA plate alternating non-carbamylated and carbamylated FCS or fibrinogen.
2. Discard blocking buffer and wash 3x with PBS/0.05% Tween-20.
3. Dilute sera 1:100 in PBS/3% FCS
4. Add 100 uL of each sera to a non-carbamylated and carbamylated protein well-seal plate
5. Incubate 2 hr at room temp
6. Wash 3x with PBS/0.05% Tween-20.
7. Dilute anti-human IgG-HRP secondary ab 1:10000 in PBS/3% FCS.
8. Add 100 uL to each well-seal plate
9. Incubate 2 hr at room temp
10. During this incubation warm substrate to room temp (R&D Sol'n A and B)
11. Wash 4x with PBS/0.05% Tween-20
12. Prepare substrate by combining equal vol of A and B just before adding to well.
13. Add 100 uL substrate-incubate about 5 min-keep an eye on the plate-wells will begin to turn blue.
14. Add 50 uL of stop solution (2 N sulfuric acid)
15. Read plate on luminometer.