

### **Assessment of angiogenesis.**

Tissue is fixed, embedded and sectioned. Slides are trypsinized with 0.1% trypsin at 37°C for 5 min, (Difco) and incubated with 180 ml of methyl alcohol and 3 ml of 30% hydrogen peroxide, blocked with 2% rabbit serum/PBS/Triton for 2h, followed by incubation overnight with rat anti-mouse CD31 (PECAM-1) antibody (PharMingen 01951D) at 4°C. Slides are then incubated with biotinylated rabbit anti-rat antibody (Vector Laboratory, BA-4001) for 30 min, developed with ABC complex and DAB substrate, and counter stained with 0.1% nuclear fast red in 5% aluminum sulfate for 2 min. Morphometry is used to determine the average number of PECAM-1 positive vessels per high power field in each group.