

IHC CD31 PARAFFIN SECTIONS

Rehydration	1.	100%	5'
	2.	90%	5'
	3.	70%	5'
	4.	50%	5'
	5.	H2O	5'
	6.	1X PBS	5'
inhibition endogenous peroxidase	7.	3% H2O2 in Methanol	20'
	8.	1X PBS	1 time 5'
Antigen retrieval	9.	Proteinase K (0.1 mg/ml) in 1X PBS	15'
	10.	1X PBS	2 times 5'
Blocking	11.	Blocking solution 1% Rabbit Serum 0.05% Tween In 1X PBS	1 hr
	12.	1X PBS	1 time 5'
Primary antibody	13.	Primary Antibody 1/100e Rat anti-mouse CD31(PECAM) BD Pharmingen (550274)	3hrs
	14.	1X PBS	2 times 3'
Blocking	15.	Blocking solution	7'
Secondary antibody	16.	Secondary antibody 1/100e Biotinylated rabbit anti-rat IgG (H+L) Vector Laboratories (BA-4001)	45'/1hr
	17.	1X PBS	2 times 5'
Amplification	18.	ABC system	30'
	19.	1X PBS	2 times 5'
Revelation	20.	DAB (or NovaRED) revelation	5-10'
	21.	H2O	2 times 5'
counterstain	22.	Counterstain with Methyl Green (Vector)	5'/60°C
	23.	H2O	2 times 5'
Dehydration	24.	Forward steps 5 to 1 (1' each step)	
	25.	Mount with Permount	