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# Doublestaining Immunofluorescence GBP-1 / CD31

**Material:**
Rat Anti-Human GBP-1 1B1 (own antibody); IgG1
CD31 Endothelial Cell Clone JC70A; Dako #M 0823, IgG1
Alexa Fluor 488 goat anti-rat IgG; Invitrogen #A11006
Alexa Fluor 546 goat anti-mouse IgG; Invitrogen #A11030
4',6-diamidino-2-phenylindole,dihydrochloride (DAPI); Molecular Probes #D-21490
Target Retrieval Solution (TRS) pH 9,0; Dako #S 2367
Goat Normal Serum; Dianova #005-000-121
Fluorescence Mounting Medium; Dako #S3023
TBS (Tris buffered Saline = Trisbuffer) 0,05M, pH 7,6; filtered!

**Staining Protocol:**

- Remove paraffin & Rehydration:
  - 2 x 15 min Xylol (p.a.)
  - 2 x 2 min EtOH 100 % (p.a.)
  - 2 x 2 min EtOH 96 %
  - 2 x 2 min EtOH 85 %
  - 2 x 2 min EtOH 70 %

- Antigen Retrieval (Water bath):
  - Target Retrieval Solution pH 9.0 20 min 95°C (Water bath, prewarm solution)
  - Cool down at room temperature (RT), 20 min
  - 3 x 5 min Trisbuffer (TBS)

- Blocking:
  - 10% Goat Normal Serum (GNS) (diluted in PBS), 10 min, RT
  - Just dump

- Primary Antibody GBP-1 (1:10) and CD31 (1:10) in 5% GNS (diluted in PBS), 1hr, RT
  - 2 x 5 min TBS

- Secondary Antibody Alexa Fluor 488 (1:500) and Alexa Fluor 546 (1:500) in 5% GNS (diluted in PBS), 45 min, RT
  - 2 x 5 min TBS

- DAPI (1:5000) in Aqua dest., 5 min, RT
  - 2 x 5 min TBS

- Mounting

Melanie Nurtsch, 5.8.08