

**Division of Molecular and Experimental Surgery
Clinical Center of Erlangen**

Head: Prof. Dr. Michael Stürzl

Tel.: ++49-9131-85-33109

Email: Michael.stuerzl@uk-erlangen.de

Doublestaining Ki-67 / CD31

Material:

Monoclonal Mouse Anti-human Ki-67; Dako # M7240

Monoclonal Mouse Anti-human CD31; Dako # M0823

Target Retrieval Solution (TRS) pH 9,0; Dako #S 2367

Antibody Dilution Reagent, RTU; Zytomed #00-3118

EnVision™ G|2 System/AP, Rabbit/Mouse (Permanent Red); Dako # K5361

VectaMount Permanent Mounting Medium; Vector # H-5000

TBS (Tris buffered Saline = Trisbuffer) 0,05M, pH 7,6

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)

➤ EnVision™ G|2 System/AP, Rabbit/Mouse (Permanent Red)

- Vial 1, Dual Endogenous Enzyme Block, 5 min, RT
- 2 x 5 min TBS
- Primary Antibody (Ki-67) 1:80, 30 min, RT
- 2 x 5 min TBS
- Vial 2, Polymer / HRP, 10 min, RT
- 2 x 5 min TBS
- DAB+ Working Solution, 5 min, RT
- 2 x 5 min Aqua dest.
- Vial 5, Doublestain Block, 3 min, RT
- 2 x 5 min TBS
- Primary Antibody (CD31) 1:40, 30 min, RT
- 2 x 5 min TBS
- Vial 6, Rabbit/Mouse (Link)
- 2 x 5 min TBS

- Vial 7, Polymer/AP
- 2 x 5 min TBS
- Permanent Working Solution, 5 min, RT
- 2 x 5 min Aqua dest.
- Counterstain with Hematoxylin Gill III, 2 min
- Thorough rinse, 10 min
- Mounting (aqueous or permanent; if permanent: dehydrate slides, then they should be dried 30 min before mounting)

Melanie Nurtsch, 5.8.2008