**IHC, general protocol Name: Isabel Büchi**

**Category: IHC Source: C.Furer/S.Overney Version 04.November 19**

**Material:**

* TBST buffer
* Antigen Retrieval Citrate Buffer pH 6 (Sigma; C9999 )
* 3%Hydrogen Peroxide/TBS
* VECTASTAIN® Elite® ABC-HRP Reagent, R.T.U. (AdipoGen VC-PK-7100)
* 3,3′-Diaminobenzidine (DAB) Enhanced Liquid Substrate System tetrahydrochloride (Sigma D3939)

**Method:**

* Dewax Tissue: Xylol 2x 5min, 100% EtOH 2x 5min, 94% EtOH ±40 min

3min, 70% EtOH 3min, dH2O 5min

* Antigen retrieval with citrate buffer pH6 (20ml 10x+180ml dH2O;

bring to boil for 2min beforehand); Put in pressure cooker at 115°C

for 10 minutes, let cool for 25-30 min 45 min

* Rinse 2x2min with dH2O, then TBS
* Rinse 2x2min with TBS
* Block with with protein blocking solution for 30 min 30 min

Add 1°AB, incubate over night at 4° o/n

* Rinse with cold TBST 2x2min 2 min
* Add 2°AB, (HRP conjugated anti- primary species; **1:100**) incubate

for 1h RT 1h

* Rinse with TBST 2x2min 2 min
* Quench erythrocytes with 3% H2O2/TBS (17ml H2O2 35%, 10 min

fill up to 200ml with TBS) for 10min.

* Rinse with TBST 2x2min
* Detect with Sigma Fast DAB (1drop (30ul) +1 ml buffer)
* Rinse with dH2O
* Counterstain with Hematoxylin (7min Hematoxylin, rinse with ±20 min

H2O, differentiate with HCL/Alcohol 2-3 dips, “blue” in H2O for

10min, dip in 70% EtOH

* Dehydrate alcohol series (70% EtOH 1min, 94% EtOH 2x1min ±10 min

100% EtOH 3x1min, EtOH:Xylol 1min, Xylol 3x1min

* mount with Eukitt