9.006b TIMP3 and Vitronectin

* Label slide appropriately (anti-body retrieval, conc antibody etc.)
* Place small amount of tap water in the base of a slide box (with loopclasp)
* Prepare a blank slide if a negative control is required (a –ive control is required when using an antibody for the first few times to ensure that there is no contamination occurring)
* Place slides in slide rack

ANTIBODIES USED:

α-Notch3: 1E4

α-vitronetin: BV2

α-TIMP3: 13613H4

**T**ISSUE USED:

Wild-type:

* Box VI
* Patient ID: A12-113-B
* Case code: 113-12A
* Brain region: Frontal cortex arterial borderzone
* Mutation: None
* Slide numbers: 6 (A), 7 (B).

CADASIL:

* Box II
* Patient ID: R.H.
* Case Code: Unknown
* Brain region: Frontal lobe (cortex and WM)
* Mutation: R133C
* Slide numbers: 18 (C), 19 (D).

|  |  |  |  |
| --- | --- | --- | --- |
| **Slide** | **Location** | **1o antibody/dilution** | **2o antibody/dilution** |
| A | TOP | Notch3/0.005 mg/ml | α-mouse 633 |
| BOTTOM | None | α-mouse 633 |
| B | TOP | TIMP3/0.01 mg/ml | α-mouse 633 |
| BOTTOM | Vitronectin/0.01 mg/ml | α-mouse 633 |
| C | TOP | Notch3/0.005 mg/ml | α-mouse 633 |
| BOTTOM | None | α-mouse 633 |
| D | TOP | TIMP3/0.01 mg/ml | α-mouse 633 |
| BOTTOM | Vitronectin/0.01 mg/ml | α-mouse 633 |

**Day 1**

**Tissue-re-hydration + Permeabilisation + Antigen retrieval + Primary antibody incubation**

1. De-paraffinize sections in xylene 3min x 2
2. Re-hydrate tissue through graded alcohols (100%(x2)- 95%(x1) - 70%(x1)) 3 min each
3. Wash in dH2O 3 min
4. Perform antigen retrieval using pressure cooker. Fill up (200-250 ml) plastic bucket (+lid) with selected antigen retrieval reagents. 1100C 20 min.
5. Place slides into dH2O for 15 min on shaker.
6. Outline tissue with a pap pen.
7. Splitting tissue in half using a pap-pen to allow for application of different primary antibodies.
8. Load **permeabilisation solution** onto tissue for 10 minutes. Coat the other half of the tissue in **Diluent solution**.
9. Neutralize background with **blocking solution** for 2 hr @ room temp 200 ul.
10. Drain off **blocking solution**.
11. Dilute primary antibodies in **diluent solution**.
12. Incubate with 1o overnight @ 4oC.
	1. Put Dilution buffer onto the –ive control
	2. Place facing upwards in the slide box (which was earlier lined with water being careful to keep it perfectly flat

**Day 2**

1. Wash 3 x 5 min with TBS
	1. Blot end of the slides on tissue
	2. Wipe the back of the slide
2. Incubate with Secondary (20) antibody 1 hr @ room temp
	1. 633 (far red) Alexafluor 1:500
3. Wash 3 x 5 min with TBS
	1. In the second wash add DAPI if doing DAPI stain (1:1000) 5 min
4. Mount with **Vectashield Mounting Medium**.
5. Observe or store at 4°C (short term) or -20oC (long term).

**Notes:**

* Ensure the tissue does not dry out between washes as this can affect the stain quality
* When adding blocks/antibodies two slides at a time is optimum (faster, but also prevents slides from drying out)

Blocking Solution

* 10% goat serum
* 1% BSA
* TBS

Diluent solution

* 1% BSA
* TBS

Sodium citrate buffer (10 mM)

* 2.94 g tri-sodium citrate (dihydrate)
* 1 L water
* Adjust pH to 6 using HCl
* 0.5 mL Tween-20

Permeabilisation solution

* 0.2% Tween-20
* TBS