Anti-human LYVE-1 in immunohistochemistry

(by Dr Sergio Dias)

Lymphatic vessel staining in thyroid tumors, using paraffin-embedded material. After dissolving the paraffin (Xylene and ethanol washes, to re-hydrate), the immunohistochemical technique followed was quite standard. Dilution of Lyve-1 Ab used: 1:400 (overnight in humidified chamber at 4C) Secondary reagents: other commercially available secondary Abs (anti-rabbit) (60 min at RT) and subsequent steps. We used a Peroxidase-based method for the detection. The technique worked well; the lymphatic vessels can be distinguished from the remaining vessels (these identified with another marker, CD31), and are easily counted. The main concern of this technique are the re-hydration steps; the tissue(s) must never be left to dry, and the re-hydration should be done stepwise (at least 4 ethanol dilutions, 5-10 min each). For thyroid tumors, blocking with hydrogenous peroxide is also crucial.

Used protocol:

- use Paraffin embedded samples
- section thickness 3µm
- deparaffinate 3 X 5 min in Neoclear (or equivalent)
- rehydrate with alcohol (100% -96% -80% -70%, 5 min in every solution) then place in water
- block peroxidase in 1% H2O2 /methanol for 5 min.
- wash 2 x in PBS, 2x5 min in citrate-buffer / microwave 600 watt
- cool down in citrate-buffer for 15-20 min.
- wash 1 x in PBS
- Wash 2 x with PBS / 0.1%Tween
- Incubate with primary antibody (1:400 dilution) overnight (18-20 hours) at 4°C in a humidified chamber
- wash 2 x in PBS/0.1%Tween
- goat anti rabbit Ab (any source, Santa Cruz for instance) for 60 min. in humid chamber at room temperature
- wash 2 x with PBS/0.1%Tween
- reveal secondary staining with secondary reagents (any source)
- wash under streaming Distilled water (5 min)
- counter stain and mount slides