

Supplementary Table 2. Step-by-step protocol for SLFN11 immunohistochemistry with tips

Required materials

- Xylene (MUTO PURE CHEMICALS CO., LTD., 43130, #190701)
- Ethanol (MUTO PURE CHEMICALS CO., LTD., 43100, #181228)
- Epitope Retrieval Solution (pH 9.0) (Leica, #6067225)
- Methanol (Wako, #KCK3458)
- 30% hydrogen peroxide (Wako, #KCP6744)
- Foetal bovine serum (Wako, 015-21274)
- 10×TBS (Nacalai Tesque, 12748-31)
- Tween 20 (Wako, 167-11515 #APF2498)
- Mouse monoclonal anti-SLFN11 antibody (Santa Cruz, #sc-515071)
- 10×PBS (-) (Nacalai Tesque, 27575-31)
- Envision+ Single Reagents (HRP. Mouse) (Agilent Dako, K4001, #10151786)
- Simple stain DAB solution (NICHIREI CO., LTD., #H1904)
- Mayer's hematoxylin (MUTO PURE CHEMICALS CO., LTD., 88592)
- Lithium carbonate (Wako, 122-01132)
- Malinol (MUTO PURE CHEMICALS CO., LTD., 2043-2 #190116)
- Distilled water

1	Formalin-fixed paraffin-embedded tissue sections (4 mm) are deparaffinized with fresh xylene for 5 min 4 times.
2	The deparaffinized sections are rehydrated with 100% ethanol, 90% ethanol, and 80% ethanol for 5 min each and then washed with tap water for 5 min.
3	Antigen retrieval is performed by microwave heating in Epitope Retrieval Solution (pH 9.0) (lot # 6067225, Leica) for 15 min in warm mode (500 W) and then for an additional 15 min in defrost mode (200 W). Note: If the solution is evaporated and the water surface goes down after 15 min, the solution needs to be replenished to completely soak the slide glasses.
4	The sections in hot solution are kept at room temperature for 30 min to cool down.
5	The sections are washed with tap water for 5 min.
6	Endogenous peroxidase activity is blocked by incubating the sections in 0.3% hydrogen peroxide/50% methanol (distilled water 100 mL + methanol 100 mL + 30% hydrogen peroxide 2 mL) for 10 min.
7	The sections are washed with tap water for 5 min.
8	The sections are blocked with 5% foetal bovine serum/1×TBS for 10 min at room temperature.
9	The sections are washed with tap water for 5 min.
10	The sections are incubated with TBST (1×TBS + 0.5% Tween 20) for 20 min.

11	The sections are incubated with mouse monoclonal anti-SLFN11 antibody (D-2, #sc-515071, Santa Cruz, 1:50 dilution in TBST) for 1 h at room temperature. Note: We use 100 mL for each section.
12	The sections are briefly washed with PBS (-) 3 times.
13	The sections are incubated with TBST for 20 min.
14	The sections are incubated with Envision+ Single Reagents (HRP. Mouse) (Agilent Dako, K4001, #10151786) for 1 h at room temperature. Note: We use 2 drops for each section.
15	The sections are briefly washed with PBS (-) 3 times.
16	The sections are incubated with TBST for 20 min.
17	The sections are incubated with Simple stain DAB solution (NICHIREI CO., LTD., #H1904) for 10 min. Note: We use 2 drops for each section.
18	The sections are washed with tap water for 5 min.
19	The sections are counterstained with Mayer's hematoxylin for 40 sec.
20	The sections are washed with tap water for 1 min.
21	The sections are incubated with 1% lithium carbonate/distilled water (WAKO 122-01132) for 10 sec.
22	The sections are washed with tap water for 5 min.
23	The stained sections are placed in 80% ethanol, 90% ethanol, and 100% ethanol for 5 min each.
24	The stained sections are dehydrated with fresh xylene 3 times.
25	The sections are mounted with coverslips using Malinol mounting medium.