**4 lames de Ganglions et 4 poumon test**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primary Antibodies** | **Concentration** | **Dilution Ab** | **Working concentration** | **Polymer** |
| **Anti-podoplanin antibody [D2-40] (Dako)** |  | **1/100** |  | **Cocktail IgG, IgG2a, IgG2b, IgM mouse** |

**2) Antigen Retrieval Put slides**, pH **High**. Heat to 97°C, 30mn / Wash Buffer / TBS 1X

**3) LEICA staining:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Category** | **Reagent name** | **Code** | **Incubation time (mn)** | **Nombre de lames** | **Volume réactif** | **Volume diluent** | **Volume total** |
| **End Enzyme Block** | **Hydrogen peroxide 3% 10 vol** | Gilbert | **15** | 4 | 300µL | 600µL | 900µL |
| **Protein Block** | **Protein Block** | Enzo | **30** | 4 | 900µL | | |
| **Isotype** | **Cocktail IgG, IgG2a, IgG2b, IgM mouse** |  |  | 2 | 600µL | | |
| **Primary antibodies** | **D2-40** | Dako | **30** | 4 | 6µL | 594µL | 600µL |
| **Secondary antibodies** | **Polymer anti-mouse HRP** | Enzo | **30** | 4 | 900µL | | |
| **Chromogen** | **DAB** | Dako | **10 (pour les 2 tissus)** | 4 | 1gouttes | 1mL | 1mL |
| **Contrestaining** | **Hematoxyline** | Dako | **5** | 4 | 900µL | | |

Montage lame et lamelle avec glycergel

**Remarque : Ok sur le poumon**