**4 lames de Ganglions et 4 lames de poumon (18H9636) test :**

|  |  |
| --- | --- |
| **Poumon** | **Gangion** |
| **Lames\_2AR (Antigen Retrieval)** | **Lames\_2AR (Antigen Retrieval)** |
| **Lames\_4AR (Antigen Retrieval)** | **Lames\_4AR (Antigen Retrieval)** |
| **Lames\_6AR (Antigen Retrieval)** | **Lames\_6AR (Antigen Retrieval)** |
| **Isotype** | **Isotype** |

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

**1) Calculate and preparation of the reagent.** (150 µL/slide + 300 µL)

**2) Antigen Retrieval Put slides in the** LEICA BOND, pH **High**. Heat to 97°C, 30mn.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Category** | **Reagent name** | **Code** | **Incubation time (mn)** | **Nombre de lames** | **Volume réactif** | **Volume diluent** | **Volume total** |
| **Rinse** | **H202** |  | **/** | 8 | 500µL | 1000µL | 1500µL |
| **Antibody Block** | **Antibody Blocker** | Enzo | **30** | 8 | 1500µL | | |
| **Isotype** | **Cocktail IgG, IgG2a, IgG2b, IgM mouse** | Dako | **30** | 2 | 600µL | | |
| **Primary antibodies** | **D2-40** | Dako | **30** | 6 | 12 | 1186µL | 1200µL |
| **Secondary antibodies** | **Polymer anti-mouse HRP** | Enzo | **30** | 8 | 1500µL | | |
| **Chromogen** | **DAB** | Dako | **10** | 8 | 2 gouttes | 2mL | 2mL |
| **Contrestaining** | **Hematoxyline** | Dako | **5** | 8 | 1500µL | | |

3) Creating protocol on LEICA software.

Montage lame et lamelle avec glycergel

**Remarque :** Le D2-40 fonctionnait bien 2AR et en 4AR.

Signal trop faible en 6AR.