**4 lames de poumon (18H9636) test.**

|  |  |
| --- | --- |
| **CD31** | **PNAD** |
| **Iso\_CD31** | **Iso\_PNAD** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primary Antibodies** | **Concentration** | **Dilution Ab** | **Working concentration** | **Polymer** |
| **PNAd clone MECA-79 553863, BD** | **500µg/mL** | **1/100** | **5µg/mL** | **Poly rat HRP** |
| **Anti-CD31 antibody [JC/70A] (ab9498)** | **1mg/mL** | **1/100** | **10µg/mL** | **Mouse HRP Enzo** |

**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** |  | **/** | 4 | 300µL | 600µL | 900µL |
| **Isotype (for PNAD)** | **IF diluent/Isotype rat** |  | **30** | 1 | 450µL | | |
| **Primary antibodies** | **PNAd** | BD | **30** | 1 | 5µL | 495µL | 500µL |
| **Secondary antibodies (for PNAD)** | **Polymer anti-rat HRP** | Vector laboratories | **30** | 2 | 600µL | | |
| **Isotype (for CD31)** | **Cocktail IgG, IgG2a, IgG2b, IgM mouse** | Dako | **30** | 1 | 450µL | | |
| **Primary antibodies** | **CD31** | Abcam | **30** | 1 | 5µL | 495µL | 500µL |
| **Secondary antibodies** | **Polymer anti-mouse HRP** | Enzo | **30** | 4 | 900µL | | |
| **Fluorescence** | **TSA (OPAL)** |  |  | 4 | 9µL | 892µL | 900µL |
| **staining** | **DAPI** |  |  | 4 | 4 gouttes pour 2mL | | |

3) Creating protocol on LEICA software.

Montage lame et lamelle avec glycergel

**Remarque :**