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

|  |  |
| --- | --- |
| **PNAD** | **PNAD\_iso** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primary Antibodies** | **Concentration** | **Dilution Ab** | **Working concentration** | **Polymer** |
| **PNAd clone MECA-79 553863, BD** | **500µg/mL** | **1/100** | **5µg/mL** | **Poly rat HRP** |

**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** |  | **/** | 2 | 200µL | 400µL | 600µL |
| **Antibody Block** | **Antibody Blocker** | Dako | **30** | 2 | 600µL | | |
| **Isotype (for PNAD)** | **IF diluent** |  | **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 | | |
| **Fluorescence** | **TSA (OPAL)** |  |  | 2 | 6µL | 594µL | 600µL |
| **staining** | **DAPI** |  |  | 2 | 2 gouttes pour 1mL | | |

3) Creating protocol on LEICA software.

Montage lame et lamelle avec prolong

**Remarque :**

* **Le passer au 1/150**
* **TSA à 20min**
* **Blocking à 1h**