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

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

**1) Deparrafinization Slides** 30 mn at 37°C / Clearene, 2mn \*3 / EtOH (100%, 90%, 70%, 50%), 2mn / dH2O

**2) Antigen Retrieval Put slides in the** PT-Link, pH **Low** et **High**. Heat to 97°C, 30mn / Wash Buffer / TBS 1X

**3) Manually 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** | 8 | 700µL | 1400µL | 2100µL |
| **1x Rinse à l’agitation** | **TBS Tween 0.04%** |  | **2** |  | | | |
| **Protein Block** | **Protein Block** | Dako | **30** | 8 | 200µL par lame ou 4 gouttes | | |
| **Séchage** |  |  | **/** |  | | | |
| **Primary antibodies** | **PNAd** | BD | **30** | 4 | 8µL | 792µL | 800µL |
| **2x Rinse (agitation)** | **TBS Tween 0.04%** |  | **2** |  |  | | |
| **Secondary antibodies** | **Polymer anti-rat HRP** | Vector laboratories | **30** | 4 | 200µL par lame ou 4 gouttes | | |
| **2x Rinse (agitation)** | **TBS Tween 0.04%** |  | **2** |  |  |  |  |
| **Chromogen** | **DAB** | Dako | **2min ganglion et 5min30sec lung** | 8 | 2gouttes | 2mL | 2mL |
| **1x Rinse** | **TBS Tween 0.04%** |  | **2** |  | | | |
| **1x Rinse** | **dH20** |  | **2** |  | | | |
| **Contrestaining** | **Hematoxyline** | Dako | **5** | 8 | 200-400µL/lames | | |
| **1x Rinse** | **dH20** |  | **2** |  | | | |

Montage lame et lamelle avec glycergel

**Remarque : Ok**