|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Primary Antibodies** | **Concentration** | **Dilution Ab** | **Working concentration** | **Polymer** | **TSA** |
| Mouse anti-human CD3 Clone: F7.2.38 Code: M7254 |  | **1/200** |  | **HRP kit** | **Opal 520 (20minutes)** |
| Monoclonal Mouse anti-human CD20cy Clone L26 Code: M0755 | **1mg/mL** | **1/400** | **5µg/mL** | **HRP kit** | **Opal 520 (20minutes)** |
| CD23 Abcam, Rb ab16702 |  | **1/100** |  | **HRP kit** | **Opal 690 (10minutes)** |
| **PNAd clone MECA-79 553863, BD** | **500µg/mL** | **1/100** | **5µg/mL** | **Poly rat HRP** | **Opal 570 (10minutes)** |
| Ki-67 clone MIB-1 |  | **1/200** |  | **HRP kit** | **Opal 480 (10minutes)** |
| **Cytokeratin Clones AE1/AE3 M3515** |  | 1/500 |  | **HRP kit** | **Opal 780 (10minutes)** |

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

**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** |  | **/** | 1 x 6 | 400µL | 800µL | 1200µL |
| **Antibody Block** | **Antibody Blocker** | Enzo | **30** | 1 | 450µL | | |
| **1 antibodies** | **CD3** | M7254 | **30** | 1 | 3 µL | 597 µL | 600 µL |
| **2 antibodies** | **CD20** | M0755 | **30** | 1 | 2 µL | 798 µL | 800 µL |
| **3 antbodies** | **CD23** | Abcam | **30** | 1 | 5 µL | 495 µL | 500 µL |
| **4 antibodies** | **PNAd** | BD | **30** | 1 | 5 µL | 495 µL | 500 µL |
| **5 antibodies** | Ki-67 | MIB-1 | **30** | 1 | 3 µL | 597 µL | 600 µL |
| **6 antibodies** | **panCK** | **M3515** | **30** | 1 | 1 µL | 499 µL | 500 µL |
| **TSA1** | **Opal 520** | **Akoya** | **20** | 1 | 5 µL | 495 µL | 500 µL |
| **TSA2** | **Opal 620** | **Akoya** | **20** | 1 | 5 µL | 495 µL | 500 µL |
| **TSA3** | **Opal 690** | **Akoya** | **10** | 1 | 5 µL | 495 µL | 500 µL |
| **TSA4** | **Opal 570** | **Akoya** | **10** | 1 | 5 µL | 495 µL | 500 µL |
| **TSA5** | **Opal 480** | **Akoya** | **10** | 1 | 5 µL | 495 µL | 500 µL |
| **TSA6** | **DIG** | **Akoya** | **10** | 1 | 5 µL | 495 µL | 500 µL |
| **TSA7** | **Opal 780** | **Akoya** | **10** | 1 | 20 µL | 480 µL | 500 µL |
| **Secondary antibodies (for PNAD)** | **Polymer anti-rat HRP** | Vector laboratories | **30** | 1 | 450µL | | |
| **Secondary antibodies** | **Polymer HRP (kit)** | Akoya | **30** | 1\*5 | 1050 µL | | |
| **Staining** | **DAPI** | Dako | **10** | 1 | 1 gouttes | 500µL | 500µL |

3) Creating protocol on LEICA software.

Montage lame et lamelle avec glycergel

**Remarque :**

* **Passer CD3 d’une concentration de 1/100 à 1/200**
* **Revoir pour panCK (refaire la manip)**
* **Améliorer le PNAd en IF simplex**
* **+ Retester ce panel sur cette lame et sur un patient**