



## Immunophenotyping of spleen, MLN and bone marrow

### Instrument configuration and panel design

| Laser | LP filter | BP Filter | T cells     | B cells     | Myeloid cells | IMPC 2  | Bone Marrow |
|-------|-----------|-----------|-------------|-------------|---------------|---------|-------------|
| 405   | -         | 450/50    | BV421       | BV421       | BV421         | BV421   | BV421       |
| 405   | 505       | 525/50    | BV510       | BV510       | BV510         | BV510   | BV510       |
| 405   | 595       | 610/20    | Qdot605     | Qdot605     | Qdot605       | -       | Qdot605     |
| 405   | 630       | 670/30    | BV650       | BV650       | BV650         | BV650   | BV650       |
| 405   | 750       | 780/60    | BV786       | BV786       | BV786         | BV786   | BV786       |
| 488   | 505       | 530/30    | FITC        | FITC        | FITC          | FITC    | AF488       |
| 488   | 685       | 710/50    | PerCP-Cy5.5 | PerCP-Cy5.5 | PerCP-Cy5.5   | -       | PerCP-Cy5.5 |
| 561   | 570       | 585/15    | PE          | PE          | PE            | PE      | PE          |
| 561   | 750       | 780/60    | PE-Cy7      | PE-Cy7      | PE-Cy7        | PE-Cy7  | PE-Cy7      |
| 640   | -         | 670/14    | APC         | AF647       | APC           | APC     | APC         |
| 640   | 690       | 730/45    | AF700       | AF700       | AF700         | AF700   | AF700       |
| 640   | 750       | 780/60    | L/D NIR     | L/D NIR     | L/D NIR       | L/D NIR | L/D NIR     |

Order of lasers: (1) Blue, 50 mW, (2) Violet, 40 mW, (3) Red, 100 mW, (4) Yellow/Green, 50 mW

#### T cell panel (compatible to IMPC panel 1) Used for: spleen and mesenteric lymph nodes

|    | Antigen      | Fluoro-chrome | Dilution | Volume/sample | Supplier    | Clone  | Cat. no    |
|----|--------------|---------------|----------|---------------|-------------|--------|------------|
| 1  | CD45         | Qdot605       | 1:200    | 0.25          | eBioscience | 30-F11 | 93-0451-42 |
| 2  | CD5          | BV510         | 1:200    | 0.25          | BD          | 53-7.3 | 563069     |
| 3  | TCR $\delta$ | PE-Cy7        | 1:200    | 0.25          | Biolegend   | GL3    | 118124     |
| 4  | CD161/NK1.1  | BV650         | 1:100    | 0.50          | Biolegend   | PK136  | 108735     |
| 5  | CD4          | BV786         | 1:200    | 0.25          | BD          | GK1.5  | 563331     |
| 6  | CD8          | AF700         | 1:200    | 0.25          | BD          | 53-6.7 | 557959     |
| 7  | CD25         | APC           | 1:100    | 0.50          | BD          | PC61   | 557192     |
| 8  | GITR         | PE            | 1:800    | 0.06          | BD          | DTA-1  | 558119     |
| 9  | CD44         | FITC          | 1:200    | 0.25          | BD          | IM7    | 553133     |
| 10 | CD62L        | PerCP-Cy5.5   | 1:200    | 0.25          | BD          | MEL-14 | 560513     |
| 11 | KLRG1        | BV421         | 1:200    | 0.25          | BD          | 2F1    | 562897     |
| 12 | Live/Dead    | NIR           | 1:2000   | separate      | Biolegend   | n/a    | 423106     |

**B cell Panel**

Used for: spleen and mesenteric lymph nodes

|    | Antigen   | Fluoro-chrome | Dilution | Volume/sample | Supplier    | Clone     | Cat. no    |
|----|-----------|---------------|----------|---------------|-------------|-----------|------------|
| 1  | CD45      | Qdot605       | 1:200    | 0.25          | eBioscience | 30-F11    | 93-0451-42 |
| 2  | IgG1      | PE            | 1:800    | 0.0625        | BD          | A85-1     | 550083     |
| 3  | B220      | AF700         | 1:200    | 0.25          | BD          | RA3-6B2   | 557957     |
| 4  | IgM       | BV786         | 1:100    | 0.5           | BD          | R6-60.2   | 564028     |
| 5  | IgD       | PerCP-Cy5.5   | 1:200    | 0.125         | Biolegend   | 11-26C.2A | 405710     |
| 6  | GL7       | AF647         | 1:1000   | 0.05          | BD          | GL7       | 561529     |
| 7  | CD95      | PE-Cy7        | 1:400    | 0.125         | BD          | JO2       | 557653     |
| 8  | CD138     | BV650         | 1:400    | 0.125         | Biolegend   | 281-2     | 142517     |
| 9  | CD5       | BV510         | 1:200    | 0.25          | BD          | 53-7.3    | 563069     |
| 10 | CD21/35   | FITC          | 1:100    | 0.5           | BD          | 7G6       | 553818     |
| 11 | CD23      | BV421         | 1:200    | 0.25          | BD          | B3B4      | 562929     |
| 12 | Live/dead | NIR           | 1:2000   | separate      | Biolegend   | n/a       | 423106     |

**Myeloid panel**

Used for: spleen and mesenteric lymph nodes

|    | Antigen           | Fluoro-chrome | Dilution | Volume/sample | Supplier    | Clone    | Cat. no    |
|----|-------------------|---------------|----------|---------------|-------------|----------|------------|
| 1  | CD45              | Qdot605       | 1:200    | 0.25          | eBioscience | 30-F11   | 93-0451-42 |
| 2  | CD11c             | BV786         | 1:200    | 0.25          | BD          | HL3      | 563735     |
| 3  | CD11b             | BV510         | 1:800    | 0.06          | Biolegend   | M1/70    | 101245     |
| 4  | F4/80             | PerCP-Cy5.5   | 1:800    | 0.06          | Biolegend   | BM8      | 123128     |
| 5  | Ly6C              | AF700         | 1:200    | 0.25          | BD          | AL-21    | 561237     |
| 6  | Ly6G              | APC           | 1:800    | 0.06          | BD          | 1A8      | 560599     |
| 7  | CD103             | PE            | 1:200    | 0.25          | BD          | M290     | 557495     |
| 8  | CD317             | BV650         | 1:400    | 0.13          | Biolegend   | 927      | 127019     |
| 9  | MHCII/IA/IE       | FITC          | 1:800    | 0.06          | BD          | 2G9      | 553623     |
| 10 | CD86              | PE-Cy7        | 1:400    | 0.13          | BD          | GL1      | 560582     |
| 11 | CD3 (lin)         | BV421         | 1:100    | 0.50          | BD          | 145-2C11 | 562600     |
| 12 | CD19 (lin)        | BV421         | 1:400    | 0.25          | BD          | 1D3      | 562701     |
| 13 | CD161/NK1.1 (lin) | BV421         | 1:100    | 0.50          | Biolegend   | PK136    | 108735     |
| 14 | Live Dead         | NIR           | 1:2000   | separate      | Biolegend   | n/a      | 423106     |

**IMPC panel 2**

Used for: spleen (for compatibility with the International Mouse Phenotyping Consortium panels)

|    | Antigen     | Fluoro-chrome | Dilution | Volum/s<br>ample | Supplier  | Clone  | Cat. no |
|----|-------------|---------------|----------|------------------|-----------|--------|---------|
| 1  | CD19        | Pe-Cy7        | 1:400    | 0.13             | BD        | 1D3    | 552854  |
| 2  | CD5         | APC           | 1:200    | 0.25             | BD        | 53-7.3 | 561895  |
| 3  | Ly6G        | APC           | 1:800    | 0.06             | BD        | 1A8    | 560599  |
| 4  | Ly6C        | AF700         | 1:200    | 0.25             | BD        | AL-21  | 561237  |
| 5  | MHCII/IA/IE | FITC          | 1:800    | 0.06             | BD        | 2G9    | 553623  |
| 6  | CD11c       | BV786         | 1:200    | 0.25             | BD        | HL3    | 563735  |
| 7  | CD161/NK1.1 | BV650         | 1:100    | 0.50             | Biolegend | PK136  | 108735  |
| 8  | CD21/35     | PE            | 1:3000   | 0.02             | BD        | 7G6    | 552957  |
| 9  | CD11b       | BV510         | 1:800    | 0.06             | Biolegend | M1/70  | 101245  |
| 10 | CD23        | BV421         | 1:200    | 0.25             | BD        | B3B4   | 562929  |
| 11 | Live/Dead   | NIR           | 1:2000   | separate         | Biolegend | n/a    | 423106  |

**Bone Marrow panel**

Used for: bone marrow

|    | Antigen    | Fluoro-chrome | Dilution | Volume/<br>sample | Supplier    | Clone.    | Cat No.    |
|----|------------|---------------|----------|-------------------|-------------|-----------|------------|
| 1  | CD45       | Qdot 605      | 1:200    | 0.25              | eBioscience | 30-F11    | 93-0451-42 |
| 2  | CD43       | PerCP-Cy5.5   | 1:400    | 0.13              | Biolegend   | 1B11      | 121224     |
| 3  | GR1        | AF700         | 1:800    | 0.06              | Biolegend   | RB6-8C5   | 108422     |
| 4  | CD11b      | BV510         | 1:400    | 0.13              | Biolegend   | M1/70     | 101245     |
| 5  | B220/CD45R | Pe-Cy7        | 1:200    | 0.25              | Biolegend   | RA3-6B2   | 103222     |
| 6  | CD24       | APC           | 1:1600   | 0.03              | BD          | M1/69     | 562349     |
| 7  | CD138      | BV650         | 1:200    | 0.25              | Biolegend   | 281-2     | 142517     |
| 8  | BP1/Ly51   | PE            | 1:400    | 0.13              | BD          | BP-1      | 553735     |
| 9  | CD3        | BV785         | 1:100    | 0.50              | Biolegend   | 17A2      | 100232     |
| 10 | IgD        | AF488         | 1:800    | 0.06              | Biolegend   | 11-26c.2a | 405718     |
| 11 | IgM        | BV421         | 1:100    | 0.50              | Biolegend   | RMM-1     | 406518     |
| 12 | Live/Dead  | NIR           | 1:2000   | separate          | Biolegend   | n/a       | 423106     |