

**Materials List for:****A Fluorescence-based Lymphocyte Assay Suitable for High-throughput Screening of Small Molecules**Ahmed Fouda<sup>1</sup>, Mahasti Tahsini<sup>2</sup>, Fatemeh Khodayarian<sup>2</sup>, Fatimah Al-nafisah<sup>2</sup>, Moutih Rafei<sup>1,2,3</sup><sup>1</sup>Department of Microbiology, Infectiology and Immunology, Université de Montréal<sup>2</sup>Department of Pharmacology and Physiology, Université de Montréal<sup>3</sup>Molecular Biology Program, Université de MontréalCorrespondence to: Moutih Rafei at [moutih.rafei.1@umontreal.ca](mailto:moutih.rafei.1@umontreal.ca)URL: <https://www.jove.com/video/55199>DOI: [doi:10.3791/55199](https://doi.org/10.3791/55199)**Materials**

| Name   | Company                       | Catalog Number          | Comments  |
|--|-------------------------------|-------------------------|---|
| Nur77 <sup>GFP</sup> mice                          | The Jackson Laboratory        | Mouse strain No. 016617 | An in house colony was established at our animal facility   |
| 96 wells-U culture plates, sterile                 | VWR International             | 10062-902               | T-cell activation using the magnetic beads                  |
| 70 µm cell strainer, sterile                       | Corning Inc.                  | 352350                  | Generation of splenocytes cell suspension                   |
| 5 ml polystyrene round bottom tubes, sterile       | Corning Inc.                  | 352058                  | Generation of splenocytes cell suspension                   |
| 50 ml polypropylene conical bottom tubes, sterile  | VWR International             | 89039-656               | Generation of splenocytes cell suspension                   |
| 10 ml syringe without needle, sterile              | Becton, Dickinson and Company | 305482                  | To mash the spleen  |
| T-25 culture flask                                 | Greiner Bio-One               | 690 175                 | To incubate B cells during activation                       |
| 5 ml cell culture dish, sterile                    | Greiner Bio-One               | 627 160                 | To mash the spleen  |
| Penicillin- Streptomycin (10,000 U/ml)             | WISENT Inc.                   | 450-200-EL              | Component of the splenocyte media                           |
| RPMI 1600 with sodium bicarbonate and L- glutamine | WISENT Inc.                   | 350-002-CL              | Component of the splenocyte media                           |
| MEM non-essential amino acids                      | WISENT Inc.                   | 321-010-EL              | Component of the splenocyte media                           |
| HEPES free acid 1 M                                | WISENT Inc.                   | 330-050-EL              | Component of the splenocyte media                           |
| Sodium pyruvate solution (100mM)                   | WISENT Inc.                   | 600-110-EL              | Component of the splenocyte media                           |
| Fetal Bovine Serum (FBS)                           | WISENT Inc.                   | 080-910                 | Component of the splenocyte media and flow-cytometry buffer |
| Phosphate buffered saline (PBS)                    | WISENT Inc.                   | 311-010-CL              | Component of flow-cytometry buffer                          |
| 2-Mercaptoethanol (55 mM)                          | Thermo Fisher Scientific      | 21985-023               | Component of the splenocyte media                           |
| T-Cells isolation kit                              | Stemcell Technologies         | 19851                   | To isolate T cells  |
| B-Cells isolation kit                              | Stemcell Technologies         | 19854                   | To isolate B cells  |
| Mouse T-Activator CD3/CD28 superparamagnetic beads | Thermo Fisher Scientific      | 11452D                  | To activate T cells   |
| Cell isolation magnet                              | Stemcell Technologies         | 18000                   | To isolate T cells and remove the magnetic beads            |

|   |   |             |  |
|---|---|-------------|--|
| AffiniPure F(ab') <sub>2</sub> Fragment Goat Anti-Mouse IgG + IgM (H+L) | Jackson ImmunoResearch Laboratories, Inc. | 115-006-068 | To stimulate B cells                         |
| Recombinant Murine IL-7   | Peprotech                                 | 217-17      | To support T-cell survival during activation |
| Recombinant CD40L   | R&D Systems                               | 8230-CL/CF  | To stimulate B cells                         |
| Anti-mouse CD3 antibody   | BD Pharmingen                             | 561799      | To stain T cells for flow-cytometry          |
| Anti-mouse CD19 antibody  | BD Pharmingen                             | 553786      | To stain B cells for flow-cytometry          |
| Biomek FX <sup>®</sup>  | PerkinElmer Inc.                          | A31842      | To re-suspend cells after 24 h incubation    |
| Opera Phenix High Content Screening System                              | PerkinElmer Inc.                          | HH14000000  | To analyze GFP/Hoechst signal                |