**Immunofluorescence**

*Walker Orr, 03/11/2024*

**Permeabilize, Fix, and Stain (for Intracellular Targets)**

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| **Steps: Day One** |  | **Helpful Hints** |
| 1. Put coverslips in a 24-well plate and let them dry under the hood for around 30 minutes. |  | Cover slips are maintained in 100% ethanol. Sterilize the tweezers before use. |
| 1. Plate cells on coverslips. For RD cells, this is usually around 125,000 cells; 20,000 for a 96-well plate. |  | In my experience, around 175,000 cells tends to work better. |
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| **Steps: Day Two** |  |  |
| 1. Wash cells with warm PBS. |  |  |
| 1. If infecting with virus, incubate for 1 hour (MOI 1) with 120 ul virus. |  |  |
| 1. After 1 hour, add 400 ul half-serum media. |  |  |
| 1. Incubate cells for 7 more hours. |  |  |
| 1. Wash with warm PBS. |  |  |
| 1. Fix with 4% PFA for 30 minutes. |  |  |
| 1. Wash with PBS. |  | You can leave the cells overnight at this step. |
| 1. Add 400 ul of Permeabilization buffer. Let stand for **10 minutes.** |  | 0.1% Triton X-100 in PBS. |
| 1. Wash with PBS. |  |  |
| 1. Add 400 ul blocking buffer for **30 minutes**. |  | 1% BSA in PBS. |
| 1. Incubate with primary antibody for **1 hour** (start with 1:1000 if you’re not sure). |  |  |
| 1. Wash three times with PBS. |  |  |
| 1. Incubate with secondary antibody for **1 hour** |  | It might be a good idea to wrap the plate in tin foil to prevent photobleaching of the antibody. |
| 1. Wash three times with PBS. |  |  |
| 1. Incubate with Draq5 (1:1000) or DAPI (4 drops/mL) for **30 minutes.** |  |  |
| 1. Wash twice with PBS. |  |  |
| 1. Add one drop of prolong gold mounting media and mount the coverslip on a microscope slide. |  | Coverslips can be stored at 4C. |
| 1. Image with the EVOS microscope |  | In the hallway; Moss Lab. |
| 1. Prepare microscope slides with prolong gold mounting media, then transfer the coverslip to the slide. |  |  |
| 1. Leave slides overnight at room temperature to cure. Then, store at 4C. Bring to room temperature again before imaging. |  |  |

**Stain and Fix only (for Cell-surface targets)**

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| **Steps: Day One** |  | **Helpful Hints** |
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| 1. Plate cells on coverslips. For RD cells, this is usually around 125,000 cells; 20,000 for a 96-well plate. |  | In my experience, around 175,000 cells tends to work better. |
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| **Steps: Day Two** |  |  |
| 1. Dilute antibodies in ice-cold HBSS. |  |  |
| 1. Aspirate media and wash cells twice with ice-cold HBSS. |  |  |
| 1. Incubate with primary antibody for 30 minutes, on ice. |  |  |
| 1. Wash twice with ice-cold HBSS. |  | You can leave the cells overnight at this step. |
| 1. Incubate with secondary antibody for 30 minutes, on ice. |  |  |
| 1. Wash twice with ice-cold HBSS. |  |  |
| 1. Fix with 4% PFA at room temperature for 30 minutes. |  |  |
| 1. Wash three times with room-temperature HBSS. |  |  |
| 1. Add DAPI (4 drops /mL in room-temperature HBSS) or Draq5 (1:1000). |  |  |
| 1. Wash twice with room-temperature HBSS. |  |  |
| 1. Image with the EVOS microscope |  | In the hallway; Moss Lab. |
| 1. Prepare microscope slides with prolong gold mounting media, then transfer the coverslip to the slide. |  |  |
| 1. Leave slides overnight at room temperature to cure. Then, store at 4C. Bring to room temperature again before imaging. |  |  |

**Antibodies Tested**

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| **QVEU Index** | **Primary Antibody** | **Dilution Tested** | **Secondary Ind(ex/ices)** | **Secondar(y/ies)** | **Dilution Tested** |
|  | Mab979 | 1:1000 |  |  |  |
|  | HA | 1:1000 |  |  |  |
|  | Flag | 1:1000 |  |  |  |
|  | J2 (dsRNA) | 1:500 |  |  |  |
|  | G3BP1 | 1:100 |  |  |  |
|  | DDX21 | 1:200 |  |  |  |
| Ab06 | SCARB2 | **1:200**; 1:500 | S05 | Donkey Anti-Goat IgG Alexa 647 | 1:1000 |
| P02 | Anti-Heparan Sulfate 10E4 IgM | **1:200**, 1:100, 1:50 | Ab21 | Goat Anti-Mouse IgM mu Alexa 488 | 1:1000 |
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