

Micronuclei Assay

***Temperature may have a large influence on this assay, and poor results may follow if these guidelines are not followed**

Supplies and Reagents

- heparin solution (500 USP units heparin/mL PBS)(Sigma H3149)
- saline (0.9% sodium chloride, 5.3mM sodium bicarbonate)
 - 18.0g NaCl
 - 0.9g sodium bicarbonate
 - bring up to 2.0L with H₂O
 - pH to 7.3
- propidium iodide solution (1.25µg/mL)(Sigma 287075)
 - dilute in saline
- CD71/FITC (Invitrogen RM5301)
- RNase A (10mg/mL)(Sigma R5250)
 - dilute in saline
- methanol
- microhematocrit capillary tube with heparin coating (Globe Scientific 51608)

I. Cell Collection and Storage

1. At least a day before collecting blood, place 15mL polypropylene conical tubes, containing 2mL methanol each, in a -80°C freezer. There should be one tube per sample bled.
2. Collect about 50µL of blood using a hematocrit tube and expel into a 1.5mL Eppendorf tube containing 250µL of the heparin solution. Keep on ice.
3. Forcefully, deliver 180µL of cell suspension into the conical tube with cold methanol.
Note: When pipetting into cold methanol, do not allow pipet tip to touch side of tube or methanol.
4. Strike tube sharply several times with hand to break up any aggregates, and immediately place back into the -80°C freezer for at least 24 hours.

***Cells can be stored in cold methanol indefinitely**

II. Cell Labeling

1. Remove tube from freezer and strike sharply several times to break up any aggregates.
2. Immediately add 12mL ice-cold saline to the cells, invert to mix, and place on ice.
3. Pellet cells at 600xg for 5 minutes at 4°C.
4. Aspirate supernatant and resuspend cells in residual volume with P200 pipet.
5. Remove 10µL of cells and transfer to a FACS tube containing 90µL of staining solution (79µL saline, 10µL RNase A, 1µL CD71/FITC).
6. Incubate 30 minutes on ice and then 30 minutes at room temperature, all while protecting samples from light.
7. Add 1mL cold propidium iodide solution just prior to analysis, and keep on ice.

III. Data Analysis

1. Before acquiring data, try to compensate the machine to receive a good dot plot.
Note: This step is extremely difficult, but necessary to get a good image. The PI+ population will be extremely small, and so it will take some time along with trial and error to get adequate settings.
2. Acquire at least 500,000 events.
3. After acquisition, use FlowJo or Cell Quest to analyze the data.
4. A dot plot should be used, placing the FL1 channel (CD71/FITC) on one axis and the FL3 channel (PI) on the other axis.
5. If the machine was properly compensated, there should clearly be individual populations. Set a 4-quadrant gate on this plot and set the gates to separate all 4 populations.
6. Analyze data in quadrants.
 - a. Cells negative for propidium iodide and positive for CD71 are reticulocytes.
 - b. Cells positive for propidium iodide and positive for CD71 are micronucleated reticulocytes.
 - c. Cells positive for propidium iodide and negative for CD71 are micronucleated normochromatic erythrocytes.
 - d. Cells negative for propidium iodide and negative for CD71 are normochromatic erythrocytes.

