IF Protocol for γ-H2Ax

Reagents:

* Alexa Fluor 488 mouse Anti-H2Ax pS139, BD Biosciences cat # 560445
* 4% formaldehyde: 10 mL of 40% formaldehyde in 30 mL PBS
* 1% BSA: 250 mg BSA in 25 mL PBS
* 0.1% Triton X-100: 50 µL Triton X-100 in 49.95 mL PBS

Protocol

1. Wash coverslips in 1x PBS twice.
2. Fix cells in 4% formaldehyde for 10 min at RT.
3. Wash with 1x PBS twice.
4. Permeabilize cells in 0.1% Triton X-100 for 5 min at RT.
5. Wash with 1x PBS twice.
6. Dilute 5 µL methanolic phalloidin stock solution into 200 µL of 1% BSA in PBS for each coverslip.
7. Incubate coverslip with phalloidin staining solution for 20 min at RT.
	1. **Keep coverslips protected from light for this step and all subsequent steps.**
8. Wash with 1x PBS twice.
9. Dilute γ-H2Ax Ab 1:50 in 1% BSA (in PBS) solution.
	1. 500 µL of 1% BSA/PBS Ab dilution per coverslip
	2. \_\_\_\_ wells x 500 µL = \_\_\_\_\_\_\_\_ total volume of 1% BSA/PBS
	3. \_\_\_\_\_\_\_\_ (total volume 1% BSA/PBS) / 50 (Ab dilution) = \_\_\_\_\_\_ µL γ-H2Ax Ab
10. Incubate coverslip with staining solution overnight at 4°C.
11. Wash with 1x PBS twice.
12. Mount coverslips with ProLong Gold with DAPI.
13. Let mounted slides dry overnight (or until fully dry) at 4°C.