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🌐 Flow Cytometry-Based Gamma-H2AX Assay for Radiation Biodosimetry

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We use this protocol and it's working

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A. Lymphocytes separation and fixation:

- 1 Collect blood samples in heparinized Vacutainers (BD, USA).
- 2 Irradiate 2 ml aliquot of whole blood with the required radiation dose.
- 3 Incubate for 30 minutes (or other time) at 37°C.
- 4 Take the 2 ml whole blood and dilute (1:1) with 2ml PBS in a 15 ml tube.
- 5 Carefully layer the mix on 2 ml of Histopaque (Ficoll).
- 6 Centrifuge at 1,200 g (Eppendorf 5810 R) for 15 min at RT.
- 7 Isolate middle cloudy lymphocytes layer and wash 2-3 times with cold PBS*.
- 8 Fix lymphocytes($1-5 \times 10^6$) with 2% paraformaldehyde (PFA) for 10-20 minutes on ice. *At this stage, fixed lymphocytes can either be processed further for immunostaining or stored overnight at +4°C.*

B. γ H2AX immunostaining and flow cytometry measurement

- 9 Wash fixed lymphocytes twice with PBS*.
- 10 Incubate lymphocytes for 1 hour at 37°C with primary antibodies (anti-phospho-histone H2A.X, Ser139, clone JBW301; Millipore, CA, USA), in a 1:200 dilution in a buffer containing 1% bovine serum albumin (BSA) and 0.12 % TX-100.
- 11 Wash twice with PBS*.
- 12 Incubate with secondary antibodies (FITC-goat antimouse Ig BD Pharmingen, San Jose, CA, USA), in a 1:200 dilution in a buffer containing 1% bovine serum albumin (BSA) and 12 % TX-100, for 1 hour at 37°C.
- 13 Wash twice with PBS*.
- 14 Suspend lymphocytes in fresh 400-500 µl of PBS according to cells concentration.
- 15 Measure gamma-H2AX fluorescence in lymphocytes using flow cytometer (LSR II, Becton Dickinson, USA) and analyze the data using PC-based BD FACSDiva software. The following settings are used:
 - 15.1 FITC laser excitation of 488 nm and emission of 533 nm.
 - 15.2 A single population of lymphocytes is gated and used for mean fluorescence intensity (MFI) calculations.

15.3 Yields of γ H2AX are calculated as the ratio of mean fluorescence intensity of irradiated versus control samples.

***Note: All PBS washing step include suspending cells in 500..**

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