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Flow Cytometry-Based Gamma-H2AX Assay for Radiation Biodosimetry

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Protocol status: Working
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 37ºC.

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(1-5×10^6) with 2% paraformaldehyde (PFA) for 10-20 minutes on ice.
Atthis stage, fixed lymphocytes can either be processed further forimmunostaining or stored overnight at +4ºC.

B. γH2AX immunostaining and flow cytometry measurement

9 Wash fixedlymphocytes twice with PBS∗.

10 Incubate lymphocytesfor 1 hour at 37ºC with primary antibodies (anti-phospho-histone H2A.X, Ser139, clone JBW301; Millipore, CA, USA), in a 1:200 dilution in a buffercontaining 1% bovine serum albumin (BSA) and 0.12 % TX-100.

11 Wash twice with PBS∗.

12 Incubate withsecondary 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-H2AXfluorescence in lymphocytes using flow cytometer (LSR II, Becton Dickinson, USA) and analyze the data using PC-basedBD FACSDiva software. The following settings are used:

15.1 FITC laser excitationof 488 nm and emission of 533 nm.

15.2 A single populationof lymphocytes is gated and used for mean fluorescence intensity (MFI) calculations.

15.3 Yields of γH2AX arecalculated as the ratio of mean fluorescence intensity of irradiated versuscontrol samples.

∗Note: All PBS washing step include suspending cells in 500 µl PBS and centrifuging (Eppendorf 5810 R) at 100rpm for 10 minutes.

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