

Materials List for:

Evaluation of the Spatial Distribution of γ H2AX following Ionizing Radiation

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Materials

| Name | Type | Company | Catalog Number | Comments |
|--|--------------------|---------------|----------------|--|
| Roswell Park Memorial Institute -1640 (RPMI-1640) | Growth medium | Invitrogen | 22400071 | RPMI-1640, pH 7.4 medium supplemented with 10% (v/v) fetal bovine, 2mM L-glutamine, 20µg/ml gentamicin, 20mM (HEPES) N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid |
| Fetal Bovine Serum (FBS) | | Sigma-Aldrich | F2442 | |
| Bovine Serum Albumin (BSA) | | Sigma-Aldrich | A7906 | BSA (1%) is used to block any non-specific antibody binding. Primary and secondary antibodies are diluted in BSA. |
| PBS (without Ca ²⁺ and Mg ²⁺) | | Invitrogen | 17-517Q | |
| Trypan blue | | Sigma-Aldrich | T6146 | Used to distinguish between live and dead cells. |
| Triton X-100 | Reagent | Sigma-Aldrich | T8787 | Triton X-100 (0.1%) used to permeabilise cells. |
| Paraformaldehyde | Reagent | Sigma-Aldrich | 158127 | Paraformaldehyde (4%) used to fix cells. |
| Mouse monoclonal anti-phospho histone-H2AX antibody | Primary Antibody | EMD Millipore | 16193 | Dilution of primary antibody (1:500), in 1% BSA. |
| HistoneH3 (Mono-methyl K4) | Primary Antibody | Abcam | AB8895 | |
| Alexa Fluor 488 goat anti-mouse IgG (H+L) | Secondary Antibody | Invitrogen | 11029 | Dilution of secondary antibody (1:500), in 1% BSA. |
| Alexa Fluor 546 goat anti-rabbit IgG (H+L) | Secondary Antibody | Invitrogen | 11035 | |
| TOPRO3 | DNA Stain | Invitrogen | T3605 | TOPRO3 is a DNA dye with an Abs/Em of 642/661 nm. DAPI could be used if the confocal microscope is equipped with a 405 nm laser. |
| ProLong Gold | Anti-fade solution | Invitrogen | P36930 | This glycerol based mounting medium must be used with an oil based lense that matches its refractive index. |

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|----------------------------------|-------------------------------|--------------------------------|-----------|--|
| Polylysine slides | | Menzel-Glaser | | |
| Coverslips (22x50mm) | Coverslips | Menzel-Glaser | CS2250100 | |
| Tissue Culture Flask, Vented Cap | Culture Flask | BD Biosciences | 353112 | |
| Shandon Cytospin 4 | | Thermo Fisher Scientific, Inc. | | |
| Cytofunnels | | Shandon, Inc. | | |
| Filter Cards | | Shandon, Inc. | 353025 | |
| Coplin Jar, glass | | Grafe Scientific P/L | 1771-OG | |
| Staining Trough | | Grafe Scientific P/L | V1991.99 | |
| PAP Pen | | Zymed Laboratories, Inc. | 008877 | |
| Gammacell 1000 Elite Irradiator | Gamma Irradiator | Nordion International Inc. | | |
| Zeiss LSM 510 Meta Confocal | Confocal Microscope | | | Equipped with 3 lasers: 488 nm, 543 nm and 633 nm. |
| Metamorph | Software for Imaging analysis | Molecular Devices | | |