

An Automated Microscopic Scoring Method for the γ -H2AX Foci Assay in Human Peripheral Blood Lymphocytes

Shankari Nair¹, Samantha Cairncross², Xanthene Miles¹, Monique Engelbrecht³, Peter du Plessis¹, Julie Bolcaen¹, Randall Fisher¹, Roya Ndimba¹, Charnay Cunningham⁴, Wilner Martínez-López⁵, Christian Schunck⁶, Charlot Vandevoorde¹

¹Radiation Biophysics Division, Nuclear Medicine Department, iThemba LABS ²Department of Biotechnology, University of the Western Cape
³Department of Medical Biosciences, University of the Western Cape ⁴Division of Medical Physiology, Department of Medicine and Health Sciences, Stellenbosch University ⁵Biodosimetry Service, Instituto de Investigaciones Biológicas Clemente Estable and Academic Unit on Radiation Protection, Faculty of Medicine, University of the Republic ⁶MetaSystems Hard & Software GmbH

Corresponding Author

Charlot Vandevoorde
 cvandevoorde@tlabs.ac.za

Citation

Nair, S., Cairncross, S., Miles, X., Engelbrecht, M., du Plessis, P., Bolcaen, J., Fisher, R., Ndimba, R., Cunningham, C., Martínez-López, W., Schunck, C., Vandevoorde, C. An Automated Microscopic Scoring Method for the γ -H2AX Foci Assay in Human Peripheral Blood Lymphocytes. *J. Vis. Exp.* (178), e62623, doi:10.3791/62623 (2021).

Date Published

December 25, 2021

DOI

10.3791/62623

URL

jove.com/video/62623

Materials

Name	Company	Catalog Number	Comments
Bovine serum albumin - BSA	Merck	A3059	
Coplin Jar	Sigma	S6016	
Cover Slips (Size 24 x 50 mm)	Lasec	Glass2C29M2450Rec	
Cryogenic Vials (1.2 mL)	WhiteSci	607101	
Cytospin	Healthcare Technologies	JC370-12-L	
Cytospin Clips	Healthcare Technologies	JC302	
Cytospin filter cards	Healthcare Technologies	JC307	
Cytospin Funnels	Healthcare Technologies	JC372	
DAM-TRITC	Invitrogen	A16022	
DAPI-Fluoroshield	Sigma/Merck	F6057	
Ethanol	Kimix	ETD901	
Filter tips	WhiteSci	200ul (312012) and 1000ul (313012)	
Hydrochloric acid- HCl	Merck		
Histopaque	Sigma/Merck	10771	
lithium–heparin collection tubes	The Scientific Group	367526	
MetaSystems - Metafer	Metasystems	Azio Imager Z2: 195-041848	
NaOH	Merck	221465	
NovoPen	Leica Biosystems	NCL-PEN	
Paraformaldehyde	Sigma/Merck	158127	
Phosphate-buffered saline Tablets	Separations	SH30028,02	

Pipettes	WhiteSci	P11037 and P1033	X-tra Clipped Corner Slides are clipped at 45° angles to help reduce glass breakage.
Purified anti-H2A.X Phospho (Ser139) Antibody	Biolegend	613402 / 100 µg	
RPMI medium	WhiteSci	BE12-702F	
Triton X-100 (Octoxinol 9)	Sigma/Merck	T-9284	
Tubes (15ml)	WhiteSci	601002	
X-Tra adhesive slides – corner clipped	SMM Technologies	3800200E	