

Enhancing nuclear marker detection by immunofluorescence: impact of Triton X-100 concentration using PhenoVue antibodies.

Authors

Lucile Hurault
Laura Boudarel
Fabienne Charrier-Savournin
Revvity

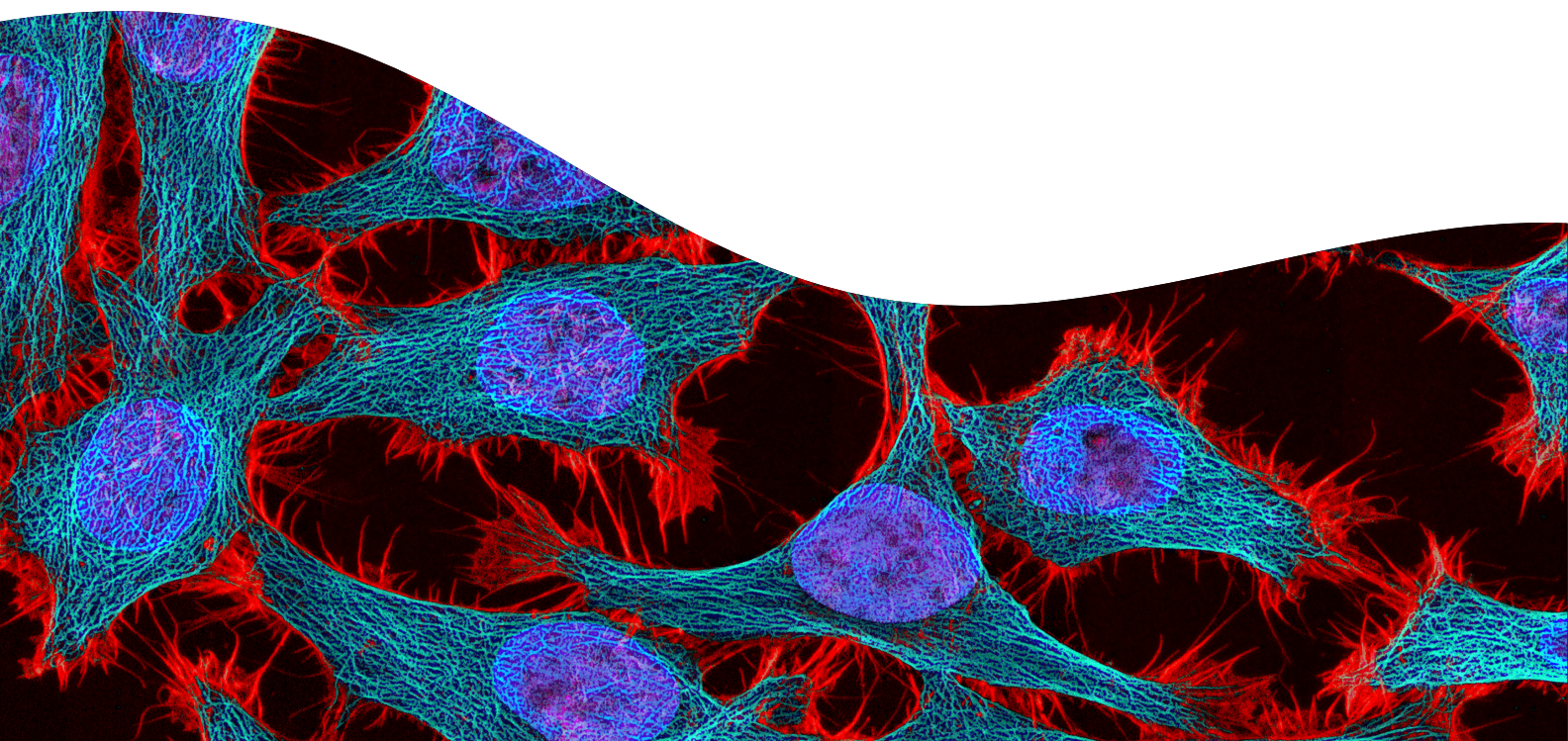
Introduction

Immunofluorescence detection of intracellular and nuclear proteins requires efficient antibody access to target epitopes. After fixation, nuclear structures may remain insufficiently accessible unless an appropriate permeabilization step is included. Detergents such as Triton X-100 are widely used for this purpose in immunofluorescence and immunocytochemistry workflows (1).

PhenoVue™ imaging reagents support robust high content imaging applications. However, as with any immunofluorescence workflow, staining quality depends on the full assay context, including fixation, permeabilization, cell type, target localization and cell density.

Nuclear markers can be particularly sensitive to permeabilization conditions. When permeabilization is insufficient, antibody penetration may be reduced, leading to weak signal, heterogeneous staining or underestimation of marker detection. This effect can become more pronounced in high density cultures, where increased cellular material and reduced reagent accessibility may limit staining efficiency.

The objective of this technical note was to evaluate whether increasing Triton X-100 concentration from 0.1 % to 0.5 % improves nuclear marker detection in fixed HeLa cells seeded at increasing densities.



Experimental overview

HeLa cells were seeded in PhenoPlate 96-well microplates at three densities: 10,000, 25,000 and 50,000 cells per well.

Cells were either left untreated or treated with etoposide to induce DNA damage. After treatment, cells were fixed with 4 % PhenoVue Paraformaldehyde methanol free and permeabilized using either 0.1 % or 0.5 % Triton X-100.

Nuclear marker detection was performed using PhenoVue 53BP1, γ H2AX and total PARP1 primary antibodies, followed by PhenoVue Fluor 647 or 488 goat anti-mouse, goat anti-rabbit, or goat anti-rat secondary antibodies, according to the protocol described in the respective product information sheets.

Nuclei were counterstained with PhenoVue Hoechst 33342 nuclear stain to evaluate cell density and nuclear morphology.

Images were acquired using an Operetta CLS™ high-content analysis system with identical acquisition settings for each marker. This allowed direct visual comparison of signal intensity, nuclear localization and staining homogeneity across cell density and permeabilization conditions.

Results

Immunofluorescence detection of 53BP1

At 10,000 cells per well, 53BP1 nuclear staining was detectable using 0.1 % Triton X-100. Under this condition, nuclear signal was visible in both vehicle and etoposide treated cells.

When cell density increased to 25,000 and 50,000 cells per well, staining obtained with 0.1 % Triton X-100 became weaker and less homogeneous. Nuclear signal was reduced despite the presence of a dense nuclear population, suggesting that the staining limitation was not due to cell loss, but rather to restricted antibody access to the nuclear compartment.

Increasing Triton X-100 concentration to 0.5 % improved 53BP1 staining at higher cell densities. Nuclear signal was stronger and more homogeneous, supporting improved permeabilization and antibody accessibility.

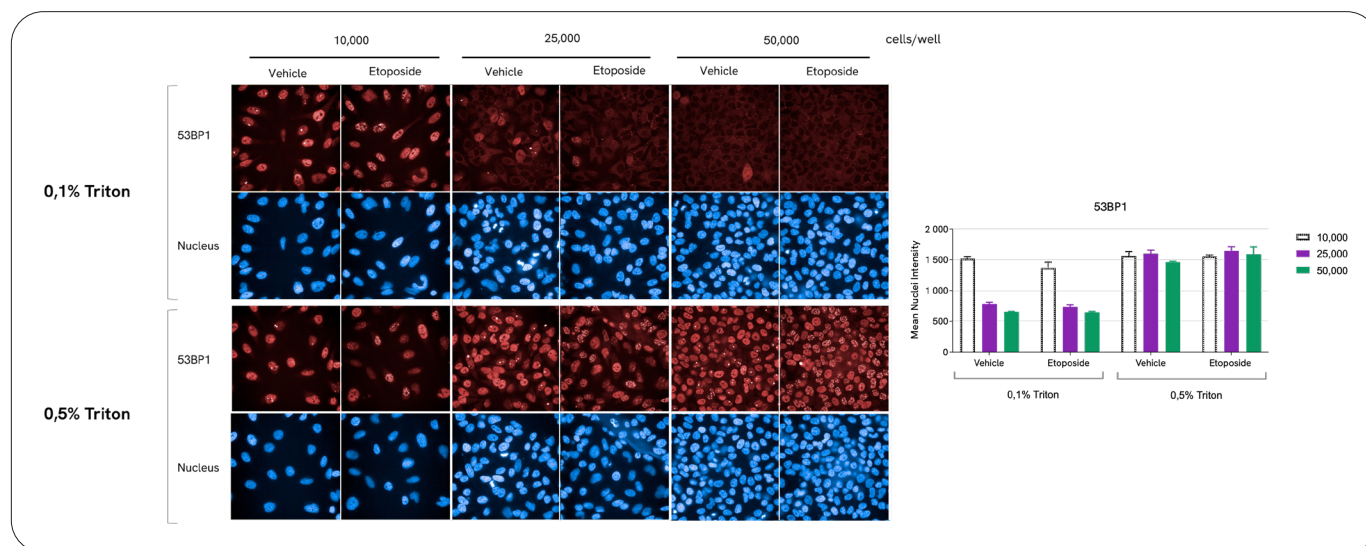


Figure 1: 53BP1 detection in vehicle and etoposide-treated HeLa cells seeded at 10,000, 25,000 or 50,000 cells per well and permeabilized with 0.1 % or 0.5 % Triton X-100. 53BP1 is shown in red and nuclei in blue.

Immunofluorescence detection of γ H2AX

γ H2AX is a nuclear DNA damage marker expected to increase following genotoxic stress. At low cell density, 0.1 % Triton X-100 allowed detection of γ H2AX signal after etoposide treatment.

At higher cell densities, γ H2AX staining was markedly reduced with 0.1 % Triton X-100. The etoposide induced signal was less clearly detected, especially at 25,000 and 50,000 cells per well.

Using 0.5 % Triton X-100 restored stronger nuclear γ H2AX staining. The difference between vehicle and etoposide treated cells was also more apparent, particularly at high cell density. This indicates that insufficient permeabilization can mask a biologically relevant nuclear marker response in dense cultures.

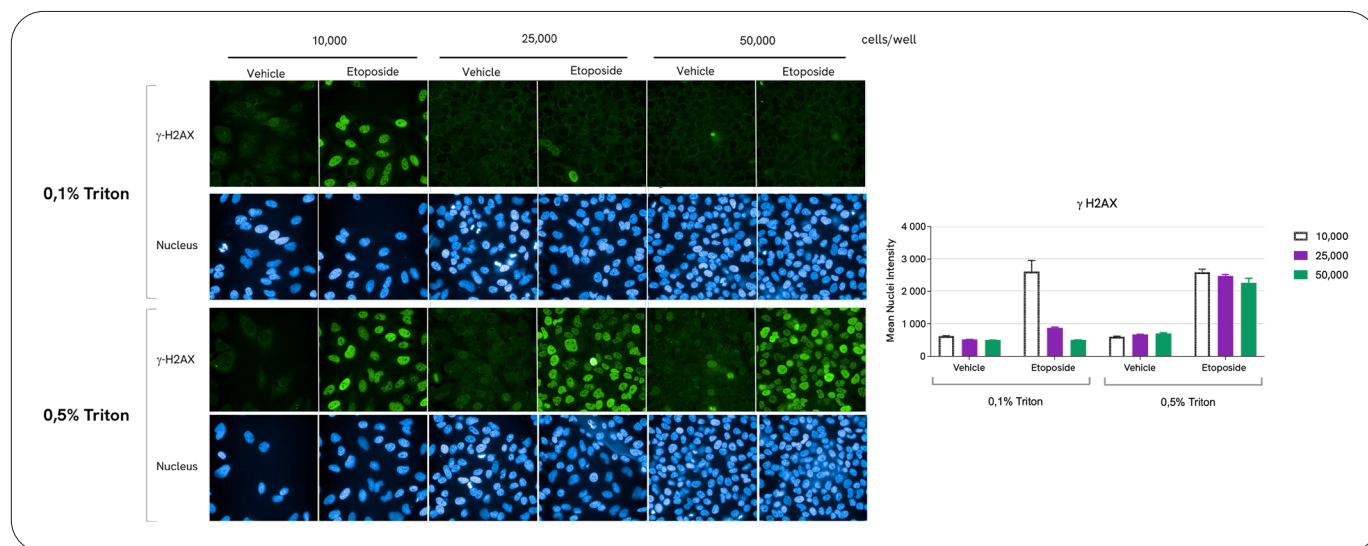


Figure 2: γ H2AX detection in vehicle and etoposide-treated HeLa cells seeded at 10,000, 25,000 or 50,000 cells per well and permeabilized with 0.1 % or 0.5 % Triton X-100. γ H2AX is shown in green and nuclei in blue.

Immunofluorescence detection of PARP1

PARP1 staining was evaluated using both mouse and rabbit PhenoVue antibodies. As PARP1 is a nuclear protein, it provides an additional marker to assess nuclear antibody accessibility.

With 0.1 % Triton X-100, PARP1 staining was detectable at 10,000 cells per well, but signal decreased at higher cell densities. At 25,000 and 50,000 cells per well, nuclear staining was weak, confirming that the effect observed with 53BP1 and γ H2AX was not restricted to a single marker.

Increasing Triton X-100 concentration to 0.5 % improved PARP1 nuclear staining for both antibody species. Signal intensity and staining homogeneity were improved, particularly under high cell density conditions.

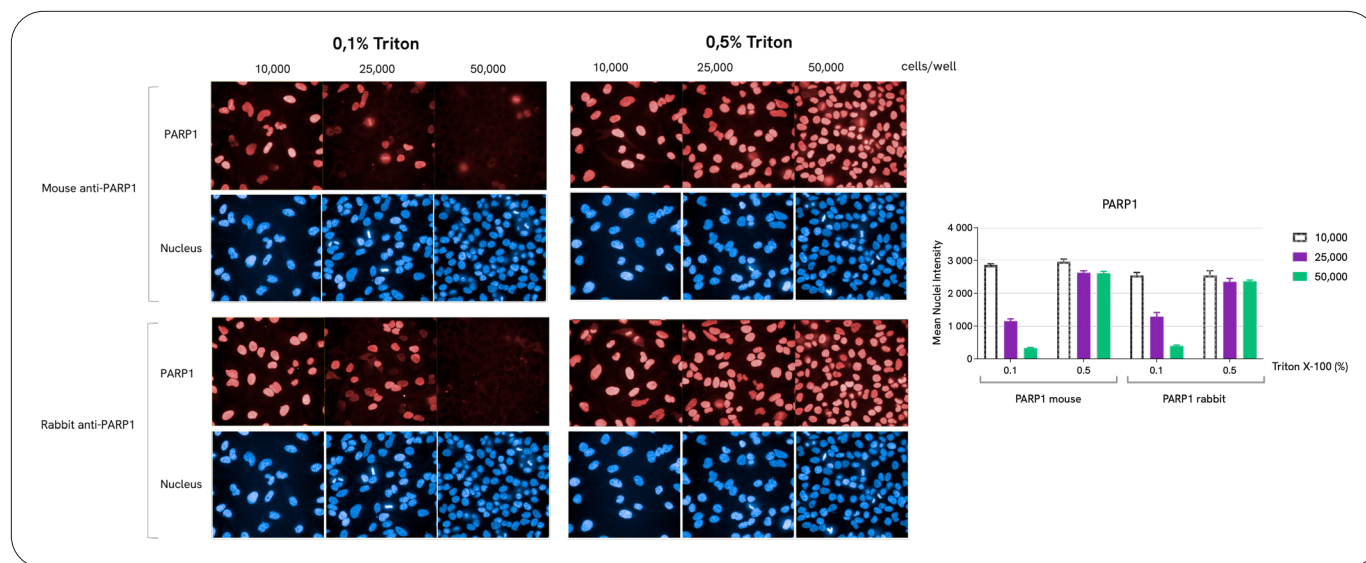


Figure 3: PARP1 detection with mouse and rabbit PhenoVue antibodies in HeLa cells seeded at 10,000, 25,000 or 50,000 cells per well and permeabilized with 0.1 % or 0.5 % Triton X-100. PARP1 is shown in red and nuclei in blue.

Discussion

These data show that cell density is a critical parameter for PhenoVue nuclear immunofluorescence workflows. While 0.1 % Triton X-100 can be sufficient at low cell density, it may become limiting when cultures reach higher confluency.

The effect was observed across several nuclear markers, including 53BP1, γ H2AX and PARP1. This supports the interpretation that reduced staining at high density with 0.1 % Triton X-100 is primarily related to insufficient permeabilization rather than poor antibody performance or lack of target expression.

The improvement observed with 0.5 % Triton X-100 is consistent with published immunofluorescence protocols describing Triton X-100 as a standard permeabilization reagent, with commonly used concentrations ranging from 0.1 % to 0.5 % depending on the target, cell type and assay format. Some immunocytochemistry protocols also recommend 0.5 % Triton X-100 for nuclear or mitochondrial protein detection.

Importantly, 0.5 % Triton X-100 should not be considered a universal condition for all markers. Triton X-100 can extract membrane associated proteins or affect cellular structures in some contexts. Permeabilization should therefore remain target and model dependent. For nuclear markers in dense cultures, however, increasing Triton X-100 concentration is a practical optimization step when weak or heterogeneous staining is observed.

Technical and practical guidance

For standard immunofluorescence assays performed at low to moderate cell density, 0.1 % Triton X-100 remains an appropriate starting condition.

At higher cell density, particularly between 25,000 and 50,000 cells per well, 0.1 % Triton X-100 may be insufficient for robust nuclear marker detection. Under these conditions, increasing Triton X-100 concentration to 0.5 % should be considered to improve permeabilization and antibody access to nuclear targets.

This adjustment is particularly relevant when nuclear staining is unexpectedly weak, heterogeneous across the well, or when an expected biological response, such as etoposide induced γ H2AX signal, is poorly detected. It is also relevant when high cell density or high confluence is required by the biological model.

As a practical approach, 0.1 % Triton X-100 can be used as the default condition for low density cultures, while 0.5 % Triton X-100 should be evaluated for dense cultures or suboptimal nuclear staining. The final condition should be confirmed for each cell type, marker and assay format, with attention to nuclear morphology, signal localization and preservation of cellular structures.

This recommendation applies primarily to nuclear markers. For membrane associated or extraction sensitive targets, milder permeabilization conditions may be required and should be validated separately.

Conclusion

Efficient immunofluorescent detection of nuclear markers depends on antibody performance as well as sample preparation conditions. Among these parameters, cell density and permeabilization efficiency can strongly influence antibody access to nuclear targets.

In this study, nuclear marker staining was evaluated in fixed HeLa cells seeded at increasing densities and permeabilized with either 0.1 % or 0.5 % Triton X-100. Three nuclear markers were assessed: 53BP1, γ H2AX and PARP1, using PhenoVue primary and secondary antibodies.

The data show that 0.1 % Triton X-100 supports nuclear marker detection at low cell density but becomes limiting as cell density increases. At 25,000 and 50,000 cells per well, nuclear staining is weaker and less homogeneous with 0.1 % Triton X-100. Increasing Triton X-100 concentration to 0.5 % improves nuclear signal intensity and staining homogeneity, helping restore reliable detection and improving visualization of biologically relevant responses, such as etoposide-induced γ H2AX signal.

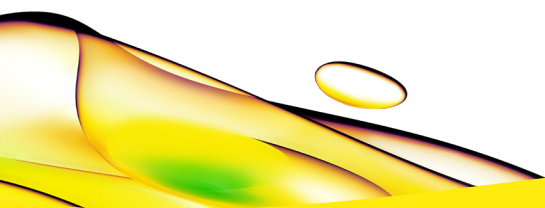
These observations support the use of 0.5 % Triton X-100 as a suitable permeabilization condition for nuclear marker detection in dense HeLa cell cultures, and highlight the importance of adjusting permeabilization conditions when working at elevated cell confluency or when weak nuclear staining is observed.

Materials

Product name	Part number	Vendor
PhenoPlate™ 96-well microplates, black, optically clear flat-bottom, poly-D-lysine-coated, lids, case of 40	6055500	Revvity
PhenoVue anti-53BP1 - Rat IgG2a Antibody, 100x	PART53BP111	Revvity
PhenoVue anti-PARP1 - mouse IgG2a antibody	PAMSPARP11	Revvity
PhenoVue anti-PARP1 - rabbit IgG antibody	PARBPARP11	Revvity
PhenoVue anti-Phospho H2AX (S139) - mouse IgG1 Antibody	PAMS139H2AX11	Revvity
PhenoVue Fluor 647 - Goat Anti-Rat Antibody Highly Cross-Adsorbed	2GXRT647H1	Revvity
PhenoVue Fluor 647 - Goat Anti-Mouse Antibody Highly Cross-Adsorbed	2GXM647H1	Revvity
PhenoVue Fluor 647 - Goat Anti-Rabbit Antibody Highly Cross-Adsorbed	2GXRB647H1	Revvity
PhenoVue Fluor 488 - Goat Anti-Mouse Antibody Highly Cross-Adsorbed	2GXM488H1	Revvity
PhenoVue Paraformaldehyde, 4 % Solution, 1x25mL	PVPFA41	Revvity
PhenoVue Permeabilization 0.5 % Triton X-100 Solution, 1x25mL	PVPERM051	Revvity
PhenoVue Dye Diluent A (5X)	PVDDA1	Revvity
PhenoVue Hoechst 33342 Nuclear Stain	CP71	Revvity
Etoposide	HY-13629	MedChemExpress

Reference

1- Piña R, Santos Díaz A I, Orta Salazar E, et al. Ten Approaches That Improve Immunostaining: A Review of the Latest Advances for the Optimization of Immunofluorescence. International Journal of Molecular Sciences. 2022;23(3):1426.



revvity