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By Emma Easthope

How In-Cell Westerns Can Advance Your Research

In-cell Westerns are a powerful and versatile technique for studying cellular behaviors at a molecular level. By combining the specificity of a traditional Western blot with the high-throughput nature of an ELISA, in-cell Westerns let researchers capture even the most subtle variations in protein expression, provided they are properly designed.

What are in-cell Westerns?

In-cell Westerns, also known as cell-based ELISAs, cytoblots, or in-cell ELISAs, are a fusion of traditional Western blots and ELISAs. They involve plating cells into 96- or 384-well microplates and carrying out any necessary experimental treatments before fixing, permeabilizing, and blocking. Proteins of interest are then detected using target-specific primary antibodies and fluorescently labeled secondaries, prior to data capture with a suitable imaging device. Usually, the secondary antibodies are labeled with near-infrared (NIR) fluorophores to minimize interference from auto-fluorescence or noise from the microplate plastic. A nuclear stain can be used to normalize the signal to the number of cells per well. The in-cell Western workflow is shown in Figure 1.

How do in-cell Westerns differ from traditional Western blotting and ELISA?

A defining feature of in-cell Westerns is that they do not call for a protein extraction step, as required by both traditional Western blotting and ELISA. Instead, in-cell Westerns detect cellular proteins *in situ*, providing more physiologically relevant results in faster time and with less risk of target antigens being lost or damaged. A further important difference between in-cell Westerns and traditional Western blotting (using enzyme-labeled antibodies and chemiluminescent detection) is that in-cell Westerns have a much higher throughput. This is because they eliminate the need to perform polyacrylamide gel electrophoresis (PAGE) and protein transfer, as well as allow for quantifying up to four different targets per well.

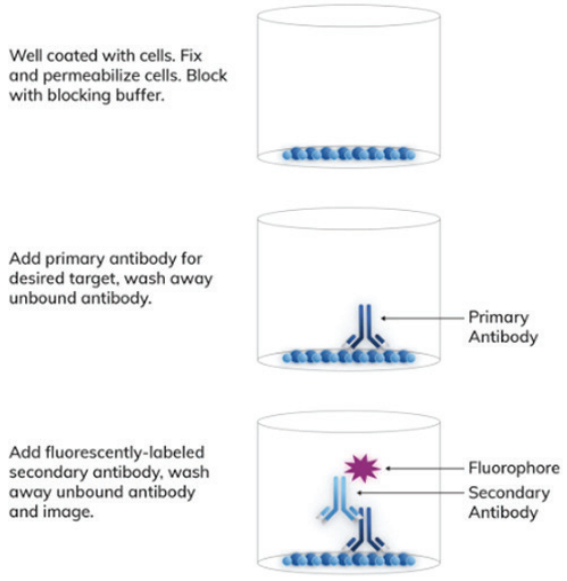

Figure 1. The in-cell Western workflow showing detection of a single protein target.

What are in-cell Westerns used for?

In-cell Westerns have utility for a broad range of research applications. These include studying the effects of small molecule drugs on cell signaling pathways, investigating changes to protein expression following genetic manipulation or post-translational modification (PTM), and assessing viral titers to understand how viruses replicate in different types of host cells. In-cell Westerns are also used for monitoring protein localization within specific cellular compartments, identifying cellular changes in disease models, and evaluating protein-protein interactions.

What do you need to perform an in-cell Western?

The reagents needed to perform an in-cell Western include the fixative and permeabilization solutions (typically, 100% methanol is used for simultaneous fixation and permeabilization), blocking solution, total stain, and both primary and secondary antibodies. For convenience, all these components (except for the primary antibodies) may be purchased as a single off-the-shelf product, such as the AzureCyto In-Cell Western Kit from Azure Biosystems. This kit is available with different NIR secondary antibody options (goat anti-mouse and goat anti-rabbit) and includes the highly sensitive AzureCyto Total Cell Stain (for incubation with primary antibodies) that makes normalization at low concentrations possible. Access to a multi-channel imaging device that is fitted with suitable lasers and detectors and is capable of bottom reading microplates is also necessary. Critically, this should feature minimal crosstalk to safeguard data quality.

Why the Sapphire™ FL Biomolecular Imager is ideal for in-cell Westerns

The Sapphire FL Biomolecular Imager is ideally suited to in-cell Westerns due to its sensitivity, speed, and high (5-micron) resolution, as well as its capacity to support multiplexed detection. The Sapphire FL is widely used for looking at total and modified (e.g., phosphorylated) versions of the same protein while also detecting an in-well standard to ensure accurate quantification across an entire plate. Figure 2 provides an example of this type of data, with the composite image demonstrating a lack of crosstalk between the signal channels.

Further advantages of the Sapphire FL include software that provides precise focal plane adjustment and an automated Z-scanning function that is used to find the focus of microplates of different brands. This affords a high level of sensitivity and specificity, as shown in Figure 3. In addition, the Sapphire FL is fitted with customizable and user-changeable laser and filter modules spanning the UV, visible, and NIR spectra that allow for easy adaptation to researchers' changing needs.

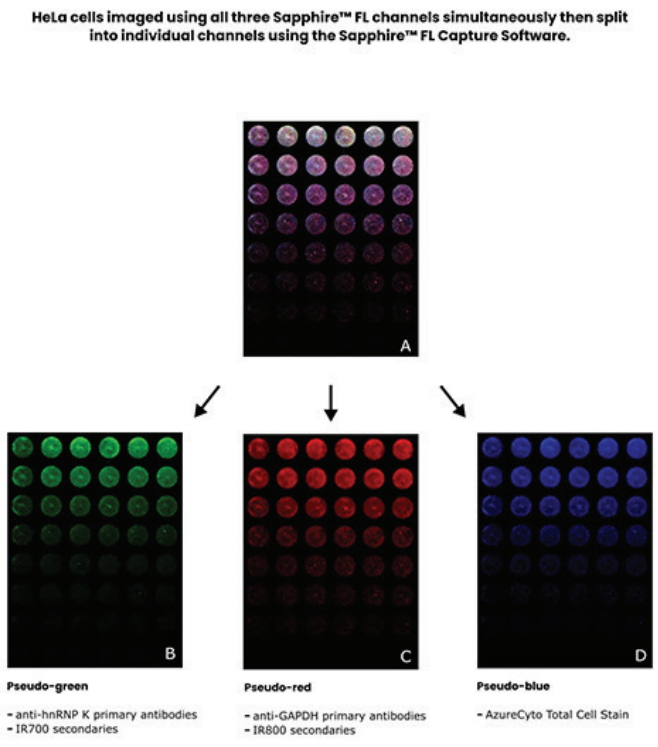

Figure 2. 1:2 serial dilution of HeLa cells seeded into a 96-well plate. (A) A composite image of three channels imaged simultaneously on the Sapphire FL at 100-micron resolution. (B) hnRNP K visualized with the 685 Standard Optical Module. (C) GAPDH visualized with the 784 Standard Optical Module. (D) AzureCyto stain visualized with the 532 Standard Optical Module.

To learn more about in-cell Westerns and the Sapphire FL Biomolecular Imager, visit azurebiosystems.com/in-cell-westerns

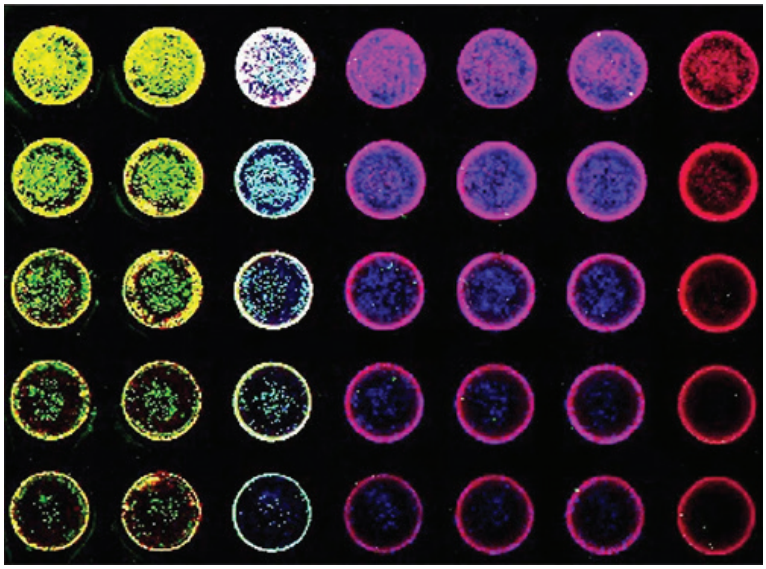

Figure 3. HeLa cells were serially diluted and seeded into a 96-well plate, cultured, fixed and permeabilized, then probed for Tubulin with AzureSpectra 550 (520 channel, green), beta-Actin with AzureSpectra 800 (785 channel, blue) and RedDot™1 Nuclear Stain as a normalization control (785 channel, red). The individual channels were scanned simultaneously then combined into a single composite image using the Sapphire Capture Software.

About the Author

Emma Easthope

Emma Easthope is the founder and director of Cambridge Technical Content Ltd, based in the U.K. Since graduating with a bachelor's degree in biology from the University of Kent at Canterbury in 2000, she has gained extensive experience developing and running immunoassays within companies including Millennium Pharmaceuticals, AstraZeneca and Cellzome. She now produces a wide range of scientific content, including regular features for Biocompare.