

AzureCyto™ In-Cell Western Kit

Accurate and Reliable In-Cell Westerns with Total Cell Normalization for Biomarker Discovery

In-Cell Western Assays combine the specificity of Western blotting with the high throughput of an ELISA. Cells are plated, fixed, and permeabilized in a 96- or 384-well plate, and targets are detected using target-specific antibodies and secondaries.

The AzureCyto In-Cell Western Kit is a complete system designed to ensure measurement accuracy and to control for well-to-well plating variability. The kit contains all reagents necessary for simultaneous detection of two biomarkers and total cells for downstream normalization.



THIS KIT OFFERS

AzureCyto Permeabilization Solution: Removes membrane lipids and facilitates permeabilization of the cell and nuclear membrane to allow the penetration of antibodies into cells for the detection of intracellular and nuclear proteins

AzureCyto Block Solution: Blocking solution formulated for cell-based assays and fluorescence detection

AzureCyto Total Cell Stain: A sensitive and linear stain that can be co-incubated with primary antibodies to streamline the detection process and reduce the number of assay steps

AzureSpectra Secondary Antibodies: For simultaneous detection of two biomarkers

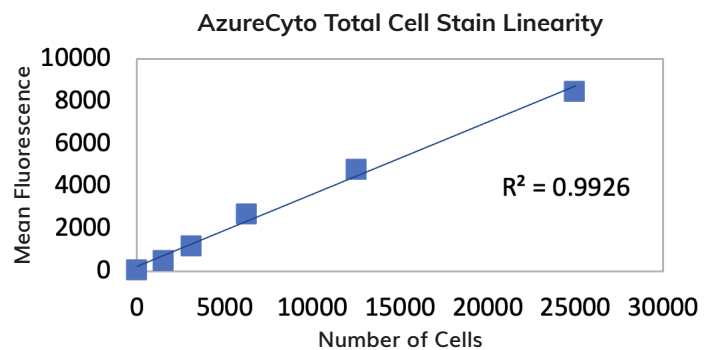
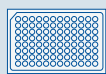


Figure 1. AzureCyto Total Cell Stain is linear over a broad range of cell volumes.

USING THE AZURECYTO IN-CELL WESTERN KIT

1



Seed cells in 96-well microplate and incubate overnight, 37°C, 5%CO₂.

2



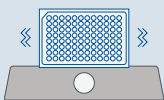
Fix, permeabilize and block

3



Incubate with primary antibodies and Total Cell Stain Solution.

4



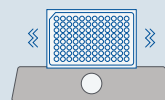
Wash away unbound primary antibody.

5



Incubate with included AzureSpectra 700 and 800 secondary antibodies.

6



Wash away unbound secondary antibody.

7



Place plate on an imaging device capable of bottom reading at 532nm, 685nm and 784nm.

WHY IN-CELL WESTERNS?

In-Cell Westerns (ICW) are a versatile assay that offers several advantages for the investigation of cellular processes and molecular events.

In situ Analysis: ICW allows for the assessment of protein expression and activation directly within the whole cell environment. This means researchers can study cellular processes without disrupting cell localization, providing physiologically relevant insights.

Quantitative Measurements: ICW enables quantitative analysis of protein levels, post-translational modifications, and signaling events. This quantitative aspect is crucial for understanding the dynamics and regulatory mechanisms of proteins within cells.

Protein Profiling: Researchers can simultaneously assess multiple proteins within the same sample using different antibodies, allowing for the study of complex signaling networks.

Biomarker Discovery: ICW is a valuable tool for biomarker discovery, where researchers can identify and validate potential biomarkers associated with diseases, cellular responses, or treatment outcomes.

High Throughput: ICW can be adapted for high-throughput screening, making it suitable for large-scale studies and drug screening applications. This efficiency accelerates the discovery of potential therapeutic targets and compounds.

UNLOCK THE TRUE POWER OF IN-CELL WESTERNS WITH AZURECYTO TOTAL CELL STAIN

Linearity: Unlock the full spectrum of cellular protein expression with AzureCyto Total Cell Stain's linear response, ensuring precise quantification and robust comparisons over a broad range of cell volumes.

Sensitivity: AzureCyto Total Cell Stain makes normalization at low concentrations possible, allowing more meaningful comparisons of small amounts of protein and uncovering subtleties of cellular signaling.

Enhanced Accuracy: Elevate your research with unparalleled accuracy, making every signal count. Normalization of both strong and weak signals over a broad linear range ensures every signal is quantified accurately.

Versatility: Tailored for a wide range of cell types and experimental conditions, AzureCyto Total Cell Stain adapts to your unique research needs.

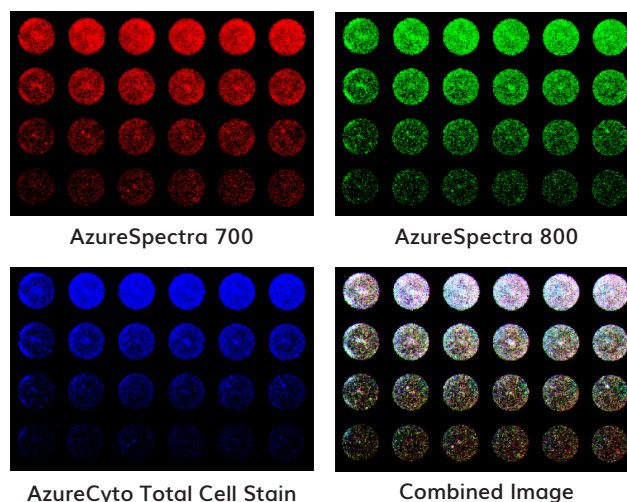


Figure 2. In-Cell Western of serially diluted cells with AzureCyto Total Cell Stain (blue) and probed for two different proteins with AzureSpectra 700 (red) and AzureSpectra 800 (green).

ORDERING INFORMATION

Part Number	Name	Description
AC2022	AzureCyto In-Cell Western Kit with AzureSpectra 700 Goat anti-Rabbit and AzureSpectra 800 Goat anti-Mouse	Includes all reagent necessary to perform 5x 96-well In-Cell Western Assays with total cell staining. Includes AzureCyto Permeabilization Solution, AzureCyto Block Solution, AzureCyto Total Cell Stain, AzureSpectra 700 Goat anti-Rabbit and AzureSpectra 800 Goat anti-Mouse secondary antibodies.
AC2023	AzureCyto In-Cell Western Kit with AzureSpectra 700 Goat anti-Mouse and AzureSpectra 800 Goat anti-Rabbit	Includes all reagent necessary to perform 5x 96-well In-Cell Western Assays with total cell staining. Includes AzureCyto Permeabilization Solution, AzureCyto Block Solution, AzureCyto Total Cell Stain, AzureSpectra 700 Goat anti-Mouse and AzureSpectra 800 Goat anti-Rabbit secondary antibodies.



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