

AdvanCell™ in-cell Western Kit

Total cell normalization and simultaneous detection of two biomarkers in whole cells

AdvanCell™ in-cell Western Kit includes all critical reagents required to generate high sensitivity whole cell staining data for five 96-well plates. AdvanCell™ Permeabilization Solution is intended to remove membrane lipids to allow antibodies to enter the cell. This solution will also permeabilize the nuclear membrane to allow for detection of nuclear proteins. AdvanCell™ Total Cell Staining Solution is a nuclear stain, provided as a 500X solution intended to be co-incubated with primary antibodies to save time and decrease the number of assay steps. Each kit includes one Spectradye IR700 and one Spectradye IR800 conjugated secondary antibody directed against mouse or rabbit primary antibodies for simultaneous detection of two biomarkers.

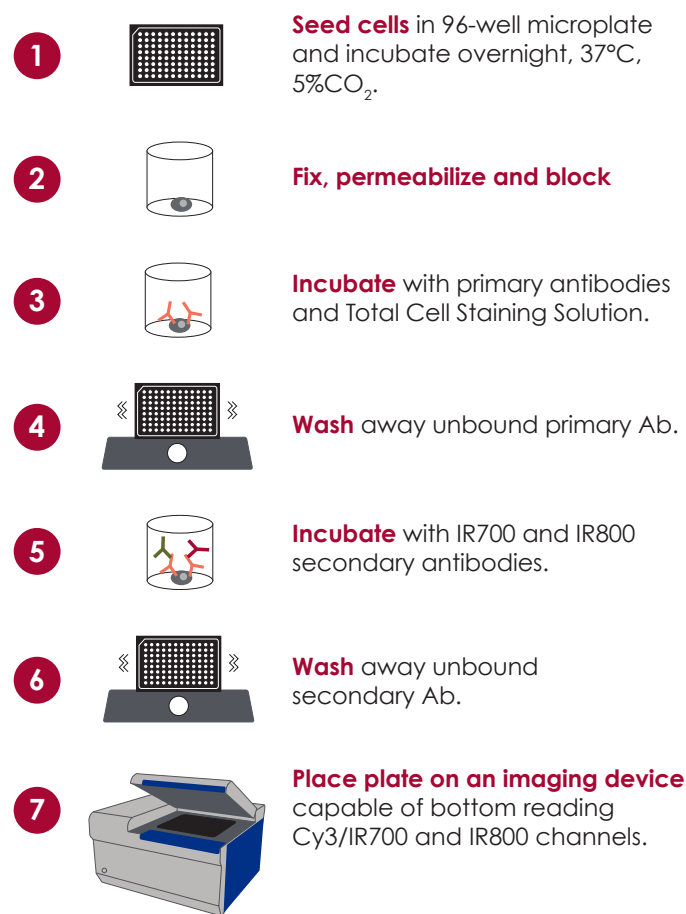


Figure 1. AdvanCell™ in-cell Western Kit workflow.

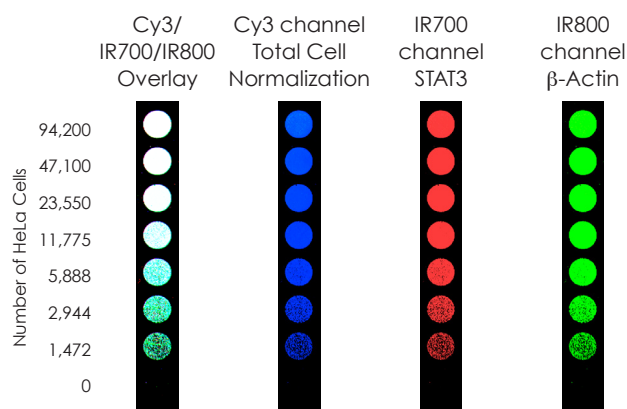


Figure 2. Quantitative and simultaneous measurement of two protein targets and total cell normalization. HeLa Cells were co-incubated overnight with AdvanCell™ Total Cell Staining Solution, 1:200 Mouse anti-STAT3 (Cell Signaling Technology #9139) and 1:200 Rabbit anti-β-Actin (Millipore #MABT523). AdvanCell Total Cell Staining Solution is a nuclear stain represented by blue and imaged in the Cy3 Channel. After washing, a 1:120 dilution of goat anti-mouse SpectraDye™ IR700, represented by red, and a 1:120 dilution of goat anti-rabbit SpectraDye™ IR800 represented by green was applied for 1 hour. After washing with 1X PBS the plate was imaged on a Sapphire Biomolecular Imager (Azure Biosystems).



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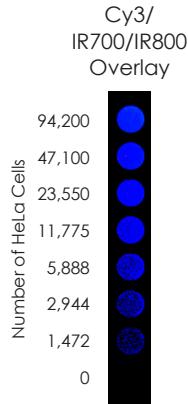


Figure 3. Cells incubated with AdvanCell™ Total Cell Staining Solution and SpectraDye Secondary antibodies do not show non-specific binding of secondary antibodies to cellular proteins.

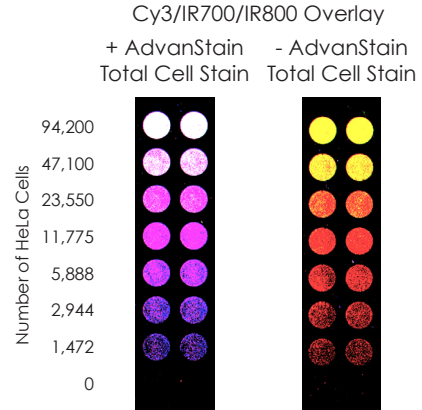


Figure 4. AdvanCell™ Total Cell Staining Solution does not interfere with primary antibody binding. HeLa Cells were co-incubated overnight with or without AdvanCell™ Total Cell Staining Solution, 1:200 Mouse anti-β Tubulin (Boster Bio #MA1112) and 1:200 Rabbit anti-EGFR (Cell Signaling Technology #4267). After washing, a 1:120 dilution of goat anti-mouse SpectraDye™ IR700, represented by red, and a 1:120 dilution of goat anti-rabbit SpectraDye™ IR800 represented by green was applied for 1 hour. After washing with 1X PBS the plate was imaged on a Sapphire Biomolecular Imager (Azure Biosystems).

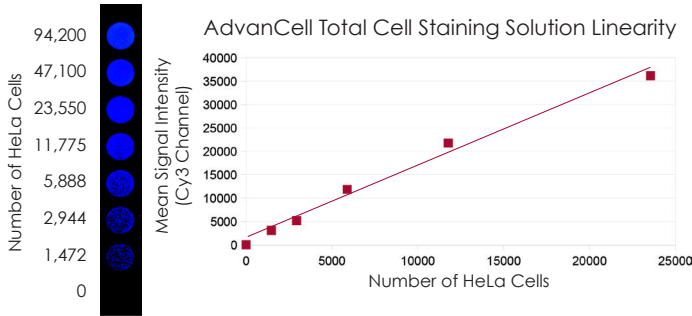


Figure 5. AdvanCell™ Total Cell Staining Solution stains cells linearly from 0–25,000 cells.

Ordering Information

Catalog Number	Product	Size
K-15065-005	AdvanCell™ in-cell Western Kit, Rb700/Ms800	5 plates
K-15066-005	AdvanCell™ in-cell Western Kit, Rb800/Ms700	5 plates



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