Troubleshooting & FAQ

AdvanCell in-cell Western Kit provides a rapid means to simultaneously stain whole cells for two biomarkers and total cell normalization. Some common questions are addressed below.

Problem	Possible Cause
High background	Excessive amount of primary antibody used. Decrease the concentration of primary antibody.
No or low signal	Insufficeint amount of primary antibody used. Increase the concentration of primary antibody.
Uneven distribution of cells	Cells are clumping or there are holes. Do not shake cells after seeding. Gently place the plate in the incubator for overnight incubation. Ensure not to touch the bottom surface of the plate with your pipette tips as this may dislodge cells from the plate.

For More Information

visit **www.products.advansta.com/Advancell-in-cell-Western** or go directly to the web-page by scanning the QR-code with your favorite bar-code scanner app on your smart phone.

Advansta Corporation
2140 Bering Drive | San Jose, CA 95131
Tel: 650.325.1980 | Fax: 650.325.1904
Email: support@advansta.com
www.advansta.com

AdvanCell™ in-cell Western Kit

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

For Catalog Numbers

K-15065-005 AdvanCell in-cell Western Kit, Rb700/Ms800

K-15066-005 AdvanCell in-cell Western Kit, Rb800/Ms700

Description

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.

advansta

August 1, 2023 | D-26058-405

Kit Contents

- R-03166-C30, AdvanCell Cell Permeabilization Solution
- R-03729-C60, AdvanCell Blocking Solution
- R-03168-060, 500X AdvanCell Total Cell Staining Solution
- R-05054-250, Goat anti-Rabbit IgG (IR700)
- R-05061-250, Goat anti-Mouse IgG (IR800)

OR

- R-05060-250, Goat anti-Rabbit IgG (IR800)
- R-05055-250, Goat anti-Mouse IgG (IR700)

Additional Materials Required

- 96-well tissue culture plates (Advansta: L-07096-005)
- Methanol (Fisher Scientific: A452SK-4)
- 1X PBS
- Plate Shaker
- Bottom Reading Imaging System with lasers/filters compatible with Cy3/IR700 and IR800 dyes

Storage Information

- The AdvanCell Cell Permeabilization Solution and AdvanCell Blocking Solution are stable at 2–8°C for one year.
- The 500X AdvanCell Total Cell Staining Solution and SpectraDye secondary antibodies are stable at -20°C for one year.

Warnings and Precautions

- The AdvanCell in-cell Western kit is for research use only.
- Always wear gloves when handling materials.
- Refer to SDS for additioanl safety information
- The product is guaranteed to be free of manufacturer defects, and to function as described when the provided protocol is followed by properly trained personnel.

Preparation of Solutions

- **1X AdvanCell Total Cell Staining Solution**: To 6mL of AdvanCell Blocking Solution add 12µL of 500X AdvanCell Total Cell Staining Solution. Add to this solution primary antibodies at the dilution factor/final concentration recommended by the antibody manufacturer. Typical dilutions range from 1:50–1:200.
- **1:120 Secondary Antibody Solution:** To 6mL of AdvanCell Blocking Solution add 50µL of each SpectraDye Secondary Antibody Stock Solution

Short Protocol

1. Seed and treat cells as desired at a density of < 25,000 cells per well and incubate overnight at 37°C, 5%CO_2.
2. Discard media and apply 50µL of 100% Methanol to each well and incubate at -20°C for 20 minutes.
3. Discard Methanol and apply 50µL of AdvanCell Permeabilization Solution and incubate at ambient temperature for 5 minutes with orbital shaking (400RPM).
4. Discard AdvanCell Permeabilization Solution and apply 100µL of AdvanCell Blocking Solution and incubate at ambient temperature for 1 hour with orbital shaking (400RPM).
5. Discard AdvanCell Blocking Solution and add 50µL of 1X AdvanCell Total Cell Staining Solution + Primary Antibodies without shaking, and incubate overnight at 2–8°C.
6. Wash each well with 100µL of 1X PBS three times, 5 minutes per wash with orbital shaking (400RPM).
7. Discard final wash and apply 50µL of SpectraDye Secondary antibodies diluted 1:120 in AdvanCell Blocking Solution. Incubate at ambient temperature for 1 hour with orbital shaking (400RPM).
8. Wash each well with 100µL of 1X PBS three times, 5 minutes per wash with orbital shaking (400RPM).
9. Discard final wash and add 100µL of 1X PBS. Place the processed plate in an imaging system equipped to bottom read Cy3/ IR700 and IR800 channels.