AzureCyto In-Cell Western Kit

Total Cell Stain Kit for 96-well plates

Short Protocol for Catalog Number

AC2022 AzureCyto In-Cell Western Kit Rb700/Ms800

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Storage Information

Store kit reagents at the temperature indicated on the component label.

Kit Contents

Reagents for 5 x 96-well in-cell Western assays:

- S1095 AzureCyto Cell Permeabilization Solution
- S1096 AzureCyto Blocking Solution
- S1097 AzureCyto Total Cell Stain, 500x
- AC2128 AzureSpectra Goat anti-Rabbit 700
- AC2135 AzureSpectra Goat anti-Mouse 800

OR

- AC2129 AzureSpectra Goat anti-Mouse 700
- AC2134 AzureSpectra Goat anti-Rabbit 800

Additional Materials Required

- 96-well tissue culture plates
- Methanol
- 1x PBS
- Plate shaker
- Plate shaker
- Bottom reading imaging system or laser scanner compatible with IR700 and IR800 dyes

Warnings and Precautions

- The AzureCyto In-Cell Western Kit is for research use only.
- Wear protective clothing such as protective glasses and gloves when handling materials.
- Refer to appropriate SDS or safety statement document for more information.

Preparation of Solutions

- **1x AzureCyto Total Cell Stain:** To 6mL of AzureCyto Blocking Solution add 12uL of 500x AzureCyto Total Cell Stain.
  - Add primary antibodies to this solution at the dilution factor recommended by the antibody manufacturer. Typical dilutions range from 1:50 – 1:200.
- **1:120 Secondary Antibody Solution:** To 6mL of AzureCyto Blocking Solution add 50uL of each AzureSpectra Secondary Antibody.

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 AzureCyto Permeabilization Solution and incubate at ambient temperature for 5 minutes with orbital shaking (400RPM).
4. Discard AzureCyto Permeabilization Solution and apply 100µL of AzureCyto Blocking Solution and incubate at ambient temperature for 1 hour with orbital shaking (400RPM).
5. Discard AzureCyto Blocking Solution and add 50µL of 1X AzureCyto Total Cell Stain + 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 AzureSpectra Secondary antibodies diluted 1:120 in AzureCyto 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 IR700 and IR800 channels.

Troubleshooting & FAQ

AzureCyto In-Cell Western Kit allows for total cell staining and normalization while simultaneously detecting two different biomarkers. Some frequently asked questions are addressed below:

Problem	Possible Solutions
High background	Excessive use of primary antibody. Decrease concentration of primary antibody.
No or low signal	Insufficient primary antibody. Increase concentration of primary antibody.
Uneven distribution of cells	Cells may be clumping. Do not shake cells after seeding. Place plates in the incubator gently. Avoid touching the bottom surface of the plate with pipette tips as this may dislodge cells.

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