

# Accellix N-1 and N-2 Assay

## INSTRUCTIONS FOR USE

### INTENDED USE

The Accellix N-1 and N-2 assays are intended to be used for phenotyping various cell-surface markers on the Accellix Platform, a bench-top flow cytometry system. In the process of evaluating a new panel of antibodies, the operator may wish to stain a cell surface protein which is not included in any of the standard off-the-shelf Accellix assays. For this purpose, the N-1 and N-2 assays include a cocktail of dried down antibodies which do not stain in the AF488 and/or PE channels. This enables the user to drop-in AF488 and/or PE-conjugated antibodies of their choice to the dried down antibodies. In an N-1 assay, one PE or AF488-conjugated antibody can be added. In an N-2 assay, two antibodies are added (one PE- and one AF488-conjugated antibody).

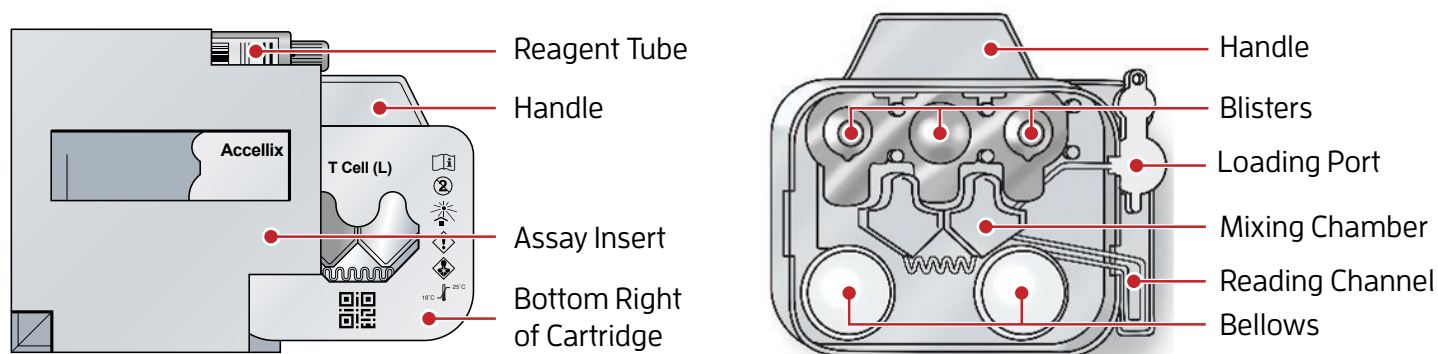
### ASSAY CONTENTS AND REQUIREMENTS

Assay Kit Contents	Equipment Required	Instrumentation Required
Assay reagent tube Assay cartridge	Calibrated pipettes and tips (100–200µL) Pulsed vortex mixer (Scientific Industries, Vortex-Genie Pulse, SKU: SI-P236) Accellix Vortex Reagent Tube Adaptor Accellix Cartridge Holder (optional)	Accellix Instrument

### ASSAY PROTOCOL

**Note:** Reagents are photosensitive. Minimize exposure to light.

1. Grip and pull the assay packaging flaps open by placing one hand on each side and pulling them apart.
2. Remove the assay insert containing the cartridge and the reagent tube in the foil pouch from the assay packaging.
3. Remove the reagent tube foil pouch and cartridge carefully from the assay insert. Avoid touching the reading channel or pressing against the assay insert as the cartridge is being removed.



4. Place the cartridge, label-side down, on a clean surface.
5. Tear open the foil pouch and remove the reagent tube. Visually check that the purple film of reagents is visible at the base of tube. If this has been dislodged, gently tap the bottom of the tube on a solid surface so the film moves back to the base of the tube. If the film remains stuck on the side of the tube, make sure to deposit sample solution with a pipette onto the film on the side of the tube instead of on the bottom of the tube (as directed in step 6), to ensure reagent is properly resuspended.
6. Uncap the reagent tube and accurately draw and transfer 40µl of well-mixed sample to the bottom of the reagent tube using an appropriate pipette. Ensure to not scrape the bottom of the tube with the pipette tip.
7. For the N-1 assay, add up to 10µl of AF488 or PE-conjugated antibody to the bottom of the reagent tube. For the N-2 assay, add up to 10µl total of conjugated antibody (one PE- and one AF-488-conjugated antibody). Dilute the antibody conjugates with PBS as needed.
8. Recap the reagent tube and place it in the Reagent Tube Adaptor of the preprogrammed pulsed vortex mixer. Ensure the tube is sitting vertically, not at an angle, and press 'Start'.

**Note:** The mixer is programmed to mix the reagent tube with the sample for 2 minutes at 1500rpm (vortex cycle = 2 seconds on, 1 second off). For more information on how to use the vortex, see the Operating Instructions for the Vortex Genie Pulse Mixer for an Accellix Assay.

**Note:** The dried reagent at the bottom of the reagent tube must be completely dissolved for optimal assay performance.

9. After the mixing program has ended, immediately uncap the reagent tube and carefully pipette the sample up and down 3 times making sure to not introduce bubbles. While holding the sample plug open, load 20µl of sample mixture into the loading port on the FMO microfluidic cartridge:

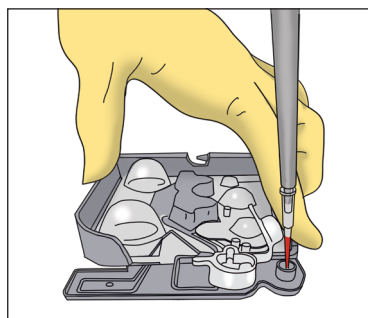


Figure A: Insert the pipette tip into the hole at the bottom of the loading port.

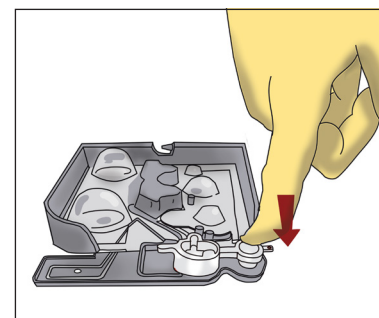



Figure B: Press the sample plug into place, covering the sample loading port.

- a. Firmly insert the end of the pipette tip into the hole at the bottom of the loading port, holding it at a 90-degree angle. Figure A.
  - b. Depress the pipette plunger to the second stop (pushing to the second stop will move the sample into the microfluidic channel connected to the loading port). Ensure the pipette tip is fully removed from the loading port before releasing your thumb.
10. With the cartridge still lying flat, firmly press the sample plug into place over the sample loading port. Figure B.
    - a. Verify that the sample fluid moved down into the channel leading to the front mixing chamber on the cartridge. Figure C.
  11. Prior to opening the instrument's loading door, initiate the assay run by tapping 'Start'  on the Accellix touchscreen. Follow the on-screen instructions to proceed.

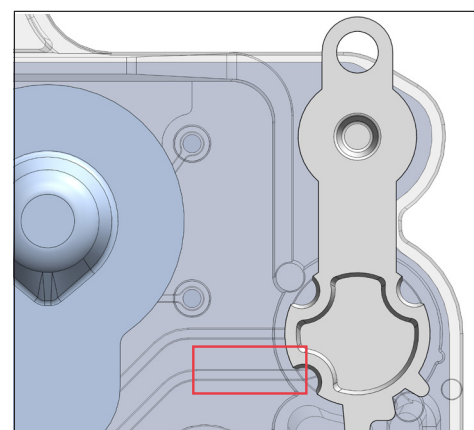


Figure C: Verify that sample fluid has moved into the microfluidic channel.

12. Hold the cartridge by its handle with the label facing away from the operator. Insert the cartridge carefully into the cartridge slot. When a slight resistance is felt, press on the right-hand corner of the cartridge using the cartridge handle. A “click” will be heard and felt, confirming the cartridge has reached the proper position. Close the Accellix Instrument door by pressing on the curved circle on the right side of the door.
13. When prompted, enter the Sample ID using the on-screen keyboard or a barcode scanner and press ‘Next’.
14. Verify the entry and press ‘Next’.
15. The assay will then proceed automatically. Total running time, including sample incubation and data analysis, is approximately 30 minutes.
16. At the conclusion of the run, follow the instructions on the screen and remove the cartridge from the Accellix Instrument.
17. The resulting data files are saved on the Accellix internal hard drive and will automatically be copied from the Accellix instrument to a storage destination configured at the time of instrument installation.



## EXPECTED RESULTS

Each assay will display results and produce a results file at the conclusion of a run. The content of the results file and any applicable result thresholds are configured and determined automatically by the Accellix instrument upon reading the barcode on the assay-specific cartridge.

## STORAGE, STABILITY AND DISPOSAL

1. Store Accellix Assays at room temperature (15–25°C) in their original packaging until ready for use.
2. Reagents are photo sensitive. Minimize exposure to light.
3. Accellix Assays are valid for use until the expiration date printed on the cartridge next to the hourglass icon and on the box.
4. Samples, used cartridges, and reagent tubes should be disposed of with proper biohazard precautions in accordance with local regulations.

## WARNINGS AND PRECAUTIONS

1. Accellix Assays are not intended for diagnosis of human or animal disease.
2. For professional use only.
3. Follow standard laboratory safety precautions when handling the Accellix Assays. Safety datasheets are available upon request.
4. Do not use a damaged cartridge, reagent tube, or Accellix Assay with damaged packaging.
5. Do not use an expired cartridge or tube. The expiration date is located on the assay packaging and component labels.
6. Do not re-use a cartridge.
7. Do not open the instrument door when an assay is in progress, as this will abort the assay and the cartridge cannot be reused.

## TROUBLESHOOTING

1. If an error is displayed on the screen of the Accellix Instrument at any point during a run, follow the instructions displayed. Please keep the cartridges in which the error occurred or take a picture of the front and back of the cartridge to share with Accellix Support.
2. If a failure occurs, or if the screen instructs to contact Accellix Support, contact:

Accellix, Inc.  
2385 Bering Drive  
San Jose, CA 95131  
email: [support@accellix.com](mailto:support@accellix.com)

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## HAZARD PICTOGRAMS



Signal Word:

Danger

Hazard Statement:

H332 - Harmful if inhaled.

H317 - May cause an allergic skin reaction.

H350 - May cause cancer.

H341 - Suspected of causing genetic defects.

## PRECAUTIONARY STATEMENTS

Prevention:

P202 - Do not handle until all safety precautions have been read and understood.

P280 - Wear protective gloves. Wear eye or face protection. Wear protective clothing.

P261 - Avoid breathing vapor.

P272 (OSHA) - Contaminated work clothing must not be allowed out of the workplace.

Response:

P308 + P313 - IF EXPOSED: Get medical attention.

P304 + P340 + P312 - IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or physician if you feel unwell.

P302 + P352 + P363 - IF ON SKIN: Wash with plenty of soap and water. Wash contaminated clothing before reuse.

P333 + P313 - If skin irritation or rash occurs: Get medical attention.

This assay contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest; do not inhale).

To learn more about the Accellix Cell Phenotyping Platform, email us at [\*\*info@accellix.com\*\*](mailto:info@accellix.com)



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