Reagent Tube Format (RTF) N-1/N-2 Assay Kit – Instructions For Use

Intended use

Each Accellix RTF Assay kit includes a microfluidic cartridge and a unitized stable dried reagent tube intended to be used for phenotyping of 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. In the N-1 assays either PE or AF488 conjugate is added, while in the N-2 assays both PE and AF488 conjugates are added. This enables the user to drop-in AF488 and/or PE conjugated wet markers of their choice to the dried down antibodies.

Applicable Products

Catalog Number	Assay Name	Description	Contents
A1001-1L	T Cell N-1 AF488 RTF Test Kit (L)	Basic T cell panel with lysis buffer for apheresis or samples requiring RBC lysis; user adds liquid AF488 conjugate antibody	CD45-PECy7, CD3-PECy5, DiYO-3 Viability, Control Beads (liquid AF488 conjugate added by user)
A1001-1NL	T Cell N-1 AF488 RTF Test Kit (NL)	Basic T cell panel without lysis buffer for purified cells or drug product; user adds liquid AF488 conjugate antibody	CD45-PECy7, CD3-PECy5, DiYO-3 Viability, Control Beads (liquid AF488 conjugate added by user)
A1001-2L	T Cell N-1 PE RTF Test Kit (L)	Basic T cell panel with lysis buffer for apheresis or samples requiring RBC lysis; user adds liquid PE conjugate antibody	CD45-PECy7, CD3-PECy5, DiYO-3 Viability, Control Beads (liquid PE conjugate added by user)
A1001-2NL	T Cell N-1 PE RTF Test Kit (NL)	Basic T cell panel without lysis buffer for purified cells or drug product; user adds liquid PE conjugate antibody	CD45-PECy7, CD3-PECy5, DiYO-3 Viability, Control Beads (liquid PE conjugate added by user)
A1001-3L	T Cell N-2 AF488 PE RTF Test Kit (L)	Basic T cell panel with lysis buffer for apheresis or samples requiring RBC lysis; user adds liquid AF488 and PE conjugate antibody	CD45-PECy7, CD3-PECy5, DiYO-3 Viability, Control Beads (liquid AF488 and PE conjugates added by user)
A1001-3NL	T Cell N-2 AF488 PE RTF Test Kit (NL)	Basic T cell panel without lysis buffer for purified cells or drug product; user adds liquid AF488 and PE conjugate antibody	CD45-PECy7, CD3-PECy5, DiYO-3 Viability, Control Beads (liquid AF488 and PE conjugates added by user)
A1004-2L	Stem Cell N-1 AF488 RTF Test Kit (L)	Basic Stem Cell panel with lysis buffer for apheresis or samples requiring RBC lysis; user adds liquid AF488 conjugate antibody	CD45-PECy7, CD34-PE, DiYO-3 Viability, Control Beads (liquid AF488 conjugate added by user)

A1004-2NL	Stem Cell N-1 AF488 RTF Test Kit (NL)	Basic Stem Cell panel without lysis buffer for purified cells or drug product; user adds liquid AF488 conjugate antibody	CD45-PECy7, CD34-PE, DiYO-3 Viability, Control Beads (liquid AF488 conjugate added by user)
A1005-2L	Stem Cell-3 N-1 AF488 RTF Test Kit (L)	Expanded Stem Cell panel with CD3 antibody and lysis buffer for apheresis or samples requiring RBC lysis; user adds liquid AF488 conjugate antibody	CD45-PECy7, CD3-PECy5, CD34-PE, DiYO-3 Viability, Control Beads (liquid AF488 conjugate added by user)
A1005-2NL	Stem Cell-3 N-1 AF488 RTF Test Kit (NL)	Expanded Stem Cell panel with CD3 antibody and without lysis buffer for purified cells or drug product; user adds liquid AF488 conjugate antibody	CD45-PECy7, CD3-PECy5, DiYO-3 Viability, Control Beads (liquid AF488 and PE conjugates added by user)

Assay Kit Contents

1. Assay reagent tube
2. Assay cartridge*

Equipment Required

1. Calibrated pipettes and tips (20-200µL)
2. Pulsed vortex mixer (Scientific Industries, Vortex-Genie Pulse, SI-P236)
3. Accellix Vortex Reagent Tube Adaptor (ASC-10085)
4. Accellix Cartridge Holder (optional)

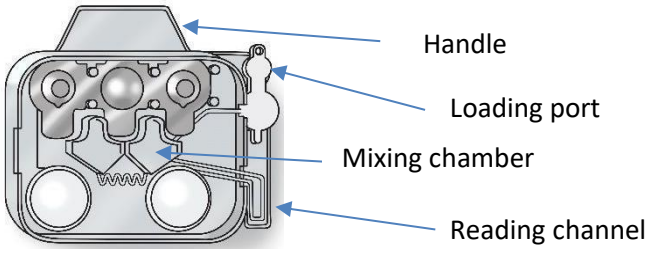
Instrumentation Required

Accellix Instrument

Assay Protocol

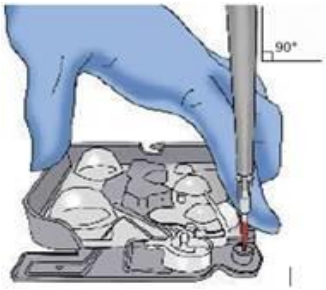
Note: Reagents are photosensitive. When working with them, ensure minimal exposure to light.

1. Open the assay kit pouch by holding it by the top edge and tearing it open from the tear notch.
2. Carefully remove the cartridge and reagent tube from the pouch, holding it by the handle.
   Note: Be sure to hold the cartridge by the handle on the top of the cartridge and avoid touching the area near the reading channel.

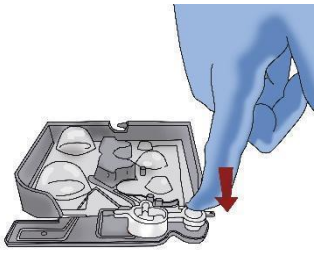


3. Place the cartridge, label-side down, on a clean surface.
4. Open the small foil pouch containing the reagent tube by tearing open from the tear notch.

5. Remove tube from pouch, uncap, and accurately pipette and transfer 40µL of the well-mixed sample to the bottom of the reagent tube.
6. Next, add the drop-in AF488 and/or PE conjugated wet antibodies/proteins to the bottom of the reagent tube. You may add up to 10 µL of the conjugate. It is recommended that you dilute the antibody conjugate with PBS if needed.
7. Recap the tube, place tube in the Reagent Tube Adaptor of the preprogrammed Pulsed vortex mixer.
   Note: The mixer is programmed to mix the reagent/sample tube for 2 minutes at 1500 rpm (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.
   Note: The reagent is dried on the bottom of the tube and must be completely dissolved for the assay to perform accurately.
8. After the mixing program ends, immediately uncap the tube and transfer 20µl of sample mixture while opening the sample plug and then load sample into the loading port on the RTF cartridge:
   - 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.



   - Depress pipette plunger to the second stop. (Pushing to the second stop will move the sample into the microfluidic channel).
9. With the cartridge still lying flat, firmly press the sample plug into place over the inlet port.



   - Verify that the sample fluid moves down into the channel leading to the front mixing chamber on the cartridge.
10. Initiate the assay run by tapping 'Start' on the Accellix touchscreen. Follow the on-screen instructions to proceed.

11. When inserting the cartridge, the side with the label should be facing away from the operator. Insert it carefully into the cartridge slot. A "click" can be heard and felt when pressing on the right-hand corner of the cartridge handle which confirms the cartridge has reached the proper position. Once the cartridge has been inserted properly, close the Accellix Instrument Door.
    Note: Another method to start a run is simply to open the door, insert a cartridge and close the door. This will bypass the "Insert cartridge and close door" screen.
12. When prompted, enter the Sample ID using the on-screen keyboard or a barcode scanner.
13. Verify the entry.
14. The assay will then proceed automatically. Total running time, including sample incubation and data analysis, is approximately 30 minutes.
15. At the conclusion of the run, follow instructions on the screen and remove the cartridge from the Accellix Instrument.
16. The resulting data files are saved on the Accellix internal hard drive and will be automatically copied from the Accellix Instrument to a storage destination. The storage destination is usually configured according to the customer's request at the time of instrument installation.

Tips and Best Practices:

1. Order the kit that fits your purpose. The N-2 kit should be used with two added liquid antibodies and will not perform properly if only one liquid antibody is added. This is due to the compensation matrix associated with each assay assuming that all the associated fluorophores are present.
2. Consider if the antibody clones that you are using may interfere with clones that are in the kit. This can be discussed further with the Accellix development team.
3. Though AF88 and FITC are excited and detected by the same laser and detector, we recommend that customers use AF488-conjugated dyes as opposed to FITC-conjugated. AF488 is more stable and less prone to photobleaching, which is more optimal for the Accellix assays.
4. When adding in commercial antibodies, first use the manufacturer's recommended volume of antibody per assay. Antibody titration is recommended.
5. When adding in a non-commercial, custom antibody, we highly recommend that customers perform a titration to identify the optimal amount of antibody that provides best results.

Storage, Stability and Disposal

1. Store Accellix assay kits at room temperature (15-25°C) in their original packaging until used.
2. Reagents are photosensitive. When working with them, assure minimal exposure to light.
3. Accellix assay kits 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.

Expected Results

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

Warnings and Precautions

1. Unless labelled otherwise, Accellix RTF Assay kits are not intended for clinical and diagnostic use.
2. For professional use only.
3. The Accellix RTF Assay kits should be handled observing standard safety precautions (do not ingest; do not inhale).
4. Do not use a damaged cartridge, tube or one with damaged packaging.
5. Do not use an expired cartridge or tube. The expiration date is located on the assay kit label.
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 will not be reusable.

Troubleshooting

1. If an error is displayed on the screen of the Accellix Instrument at any point during a run, follow the instructions displayed.
2. If a failure occurs, or if the screen instructs to contact Accellix support, contact us at the following address:

Accellix, Inc.
2385 Bering Drive
San Jose, CA 95131
email: *support@accellix.com*

Not for use in diagnostic applications

***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.

<u>Precautionary Statements</u>

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 kit 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).