invitrogen

QUICK REFERENCE

Attune NxT Flow Cytometer quick maintenance and troubleshooting guide

The Invitrogen™ Attune™ NxT Flow Cytometer is designed to require minimal maintenance. To ensure reliability of the flow cytometer, you must perform basic preventive maintenance procedures on a regular basis, as listed below.

Frequency	Procedure	Maintenance
Daily	Visual inspection	• **Check fluid levels**—Empty waste and refill fluids as needed. • **Check fluid and sensor lines**—Ensure all bottles are firmly connected. An audible click should be heard to indicate full engagement. • **Check floor of fluid bottle compartment**—Clean up any spilled liquid, remove any salt or dust deposits with a damp paper towel. If needed, decontaminate by wiping area with 10% bleach solution. Monitor, as it may indicate a leak. • **Inspect sample injection port**—Ensure it is clean and straight. • **Check Invitrogen™ Attune™ NxT sample syringe (glass syringe in left side compartment of Attune NxT Flow Cytometer)**—ensure top and bottom metal fittings are tightened; check for signs of leaks (salt deposits or rusty metal plunger). If any indications of potential leaks, replace syringe.

ThermoFisher SCIENTIFIC

Frequency	Procedure	Maintenance
Daily	Fluidics inspection	Run Startup and Performance Test • Close Attune NxT Software; then re-open Attune NxT Software and log in. • While Startup is being performed, observe syringe movements of the sample syringe and Attune NxT Autosampler. – Listen for any sounds that are out of the ordinary such as loud grinding or the absence of normal sounds during startup. Record if possible for support reference. – The sample syringe is located in the left side compartment of the cytometer. Observe for smooth movement on up/down strokes. Observe for excessive bubbles within the sample upstroke or any drops escaping past the plunger. There should be no bubbles being delivered on the upstroke of the syringe. Observe for any large air bubbles on the downstroke that might indicate a large leak or worn syringe. – The Autosampler syringe is located in the fluid bottle compartment, on the left side. Observe for the same items as listed above. • If the syringe is not moving or appears to be sticking (non-fluid motion), we recommend replacing the syringe before contacting technical support.
Daily	Cleaning	Recommended cleaning for optimal performance: • After use by researchers working with non-sticky cells, such as B and non-activated T cells – Run Sanitize SIP 2 times (~3-4 min total) – On the Instrument ribbon, click Sanitize. From the drop-down menu, select Sanitize SIP for tubes; choose the drop-down menu button for the Attune NxT Autosampler SIP to clean Autosampler if plates have been run – 1st iteration—Use 3 mL bleach, 10% – 2nd iteration—Use 3 mL Attune Wash Solution (Cat. No. A24974) • After use by researchers working with sticky cells, such as dendritic cells (DCs), red blood cells (RBCs), activated T cells, many cancer lines, bacteria, yeast – Run Quick Deep Clean cycle (~20–30 min total) • At the end of day or after the last run of the day: – Run Thorough Shutdown
Daily		Power-cycle instrument and run Autosampler calibration • Close software, turn off power on the Autosampler and flow cytometer, and wait 15–30 sec for internal boards to reset • Turn on Autosampler, remove shutdown plate, and close door • Turn on the flow cytometer • Open software and log in • Perform Startup and Performance Test

Frequency	Procedure	Maintenance
Monthly	Computer	Check space on D drive: • If running low on space (less than 50 GB free), request that users export, save externally, and delete experiments from their Experiment Explorer • To determine which accounts may be using the most data: – Check user log: Log in as system administrator; go to Instrument tab → System Log → User Log tab (bottom left). Filter by [install date] to current date; All Users; Sample Count (instead of User Time) – User folder size check in D drive: D:\\User\public\public documents\life technologies\AttuneNxT\Userdata
Monthly	Optical filters	• If there is dust, use compressed air or a bulb blower to gently blow away the dust • If there is grease (e.g., fingerprints), gently wash with mild detergent, rinse with deionized water, and air dry; do not wipe dry
Monthly	Autosampler calibration	Run Autosampler calibration every 30 days (located on The Instrument tab) • To check the last date, go to Options → Resources
Every 3–6 months (depending on usage volumes)	Fluidics decontamination	Perform System Decontamination • After System Decontamination is complete, replace both focusing fluid filters (Cat. No. 100022587) • Run 3 Startup cycles, 2 De-bubble cycles, and 2 Rinse cycles to saturate new filters with Attune Focusing Fluid (Cat. No. 4449791) and purge air from the system

Quick troubleshooting guide

Please note any issues on the Instrument Record Sheet, even if you are able to resolve them on your own. This will enable tracking of repeated issues that may indicate that additional service is needed.

Observation	Recommended solutions
Data stream on time plot looks choppy or no events are coming through	• Test for sample syringe movement—glass syringe located in left side compartment of cytometer. • While observing sample syringe, initiate Rinse function (located on Instrument tab). – If syringe is not moving at all, check to make sure the USB cable connection between the back of the cytometer and the computer is firmly connected. – Perform a power cycle of instrument—Close software and turn off cytometer and Autosampler; then turn on Autosampler, then the cytometer, then open software. Log in and perform Startup. While Startup is executing, observe sample syringe. • If syringe is still not moving or appears to be sticking (non-fluid motion), contact our technical support team. • If sample syringe movement is okay, system may have a clog.
System appears to have a clog or individual channels fail Performance Test	For additional cleaning, recommend having Contrad-70 solution on hand (Fisher Scientific, Cat. No. 04-355): • Perform Quick Deep Clean using 3 mL 50% Contrad-70 in water (instead of bleach). • If Quick Deep Clean is not sufficient, perform Thorough Deep Clean using 50% Contrad-70 in water (instead of bleach), then run Thorough Shutdown using 50% Contrad-70 in water. • If these do not solve the issue, contact our technical support team.
Performance Test: all parameters fail, or system stops before Performance Test is complete and shows message to restart/clean/de-bubble etc.	• Select Rinse on the Instrument tab. • Select Calibrate Autosampler function (on Instrument tab), then choose Cancel; probe should lift. You can then restart plate acquisition at the next well. • If the above method does not work—Turn off power to the Autosampler and flow cytometer, power on, and perform Startup. During Startup, observe smooth movement of the Autosampler probe. It should travel in a straight path up and down. If it stutters in any way, it may be bent and require replacement. • Observe for any salt buildup or appearance of focusing fluid on plates or trays. This may be indicative of a faulty waste connection. After you have confirmed a solid connection to waste, observe for droplets being deposited into the waste bottle as Startup progresses.

invitrogen

Observation	Recommended solutions
Loose connections errors—system displays an error message (flashing blue backlights) that a fluidics sensor connection is loose (e.g., wash or waste):	• Check to be sure that the bottle is full (focus fluid, wash solution, shutdown) or empty (waste). Top off all bottles and empty the waste. • Check all fluid lines and sensor connections—make sure they are firmly seated and plugged in. • Check the connections for both the cytometer and the Autosampler. • If the connections appear to be firm and the error will not clear, a new bottle or connection may be needed. Contact our technical support team.
Leak detected errors	• Leak detected errors—Both the Attune NxT Flow Cytometer and the Autosampler have a leak detector in their bottle bay basins that are designed to pick up large leaks (~5 mL). – If either leak detector is triggered, inspect to confirm that there is a leak and then clean as necessary. Look for any signs of the source, such as a loose fitting or damaged bottle connections. Tighten or replace as necessary. – If there is a sign of an intermittent leak, confirm the leak sensor is operational by placing your finger over the leak sensor face in the bottle bay. It should trigger the leak sensor. The sensor may need sensitivity adjustment. Contact our technical support team.

invitrogen

Additional troubleshooting information

- Attune NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide (Pub. No. 100024234)
- Attune NxT desktop folder “Attune NxT User Guides”
- Attune NxT desktop folder “Helpful Attune information”—includes training slides PDF, fluorophore guide, consumables list, quick reference guide

Attune NxT Flow Cytometer support contact info

For immediate assistance, please call: 1-800-955-6288 (9:00 a.m.–8:00 p.m. ET)

Option 3 and then option 1 for instrument service and hardware support

Note: You will need the Serial Number of your instrument when contacting our technical support team.

Or

Email **instrumentservices@thermofisher.com** for the technical assistance center (TAC) and instrument services

ThermoFisher SCIENTIFIC