Attune NxT Awaken Procedure After Long Term Shutdown

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I. Restart Preparation

1. Recommended consumable parts to have on-hand

    a. 100022587 FILTER, 0.2UM, MEDIAKAP-5PLUS **(x2)**

    b. 4490099 ASSY, TBG DIBA 517-022 (SIP)

    c. A43635 ATTUNE FLOW CELL CLEAN SOL

    d. 100022591 1ML Syringe (NxT syringe)

    e. 4478686 ASSY, SYR 1.0ML W/ THRD LK RC (AAS Syringe)

    f. 100054593 SYRINGE,1ML UHMWPE PISTN, C-SERIES (CytKick syringe)

    g. 100022156 ASSY, WASTE BTL1.9L

    h. 100022155 ASSY, FOCUS BTL1.9L

    i. 4477850 ASSY, BOTTLE WASTE AAS

    j. 4477847 ASSY, BOTTLE FOCUS AAS

2. Rinse bottles and use fresh reagents

    a. 4488621 ATTUNE 1 X 1L FOCUSING FLUID

    b. A24974 ATTUNE WASH SOLUTION

    c. A24975 ATTUNE 1X SHUTDOWN SOLUTION

    d. 4449754 ATTUNE PERFORMANCE TRACKING BEADS

3. Power cycle instrument(s) and reboot computer

II. If System Decontamination Script was not part of the long-term shutdown

(If system decontamination was run as part of shutdown, use option III below)

1. Run System Decontamination Script and change focusing filters *(~ 60 minutes)*
2. Run 3x Startup *(~10 minutes)*
3. Inspect syringes during startup and replace if necessary:

   Look for dried salt or anything that looks like debris in the syringe barrel and on either side of the plunger; any signs of this would drive syringe replacement.

   - NxT syringe - Cat. No. 100022591
   - Autosampler syringe Cat. No. 4478686
   - CytKick syringe Cat. No. 100054593
4. Run 2x Debubble w/ Debubble solution *(~4 minutes)*

   https://www.thermofisher.com/order/catalog/product/A10496#/A10496
5. Run 2x Rinses *(< 1 minute)*
6. Run thorough Deep Clean using Flow Cell Cleaning Solution *(~60 minutes)*

   If it is not available: freshly prepare 10% bleach solution (.5% Sodium Hypochlorite)
   https://www.thermofisher.com/order/catalog/product/A43635#/A43635
7. Run Performance Test (PT) *(~ 5 minutes)*
   - If PT test passes – continue to experiment (Similar to pre-shutdown PT)
   - If PT does not pass not, go to next step
8. Repeat Deep Clean with warm (37° C) DI Water (Milli-Q or similar) NOT 10% bleach. Do NOT use boiling water. *(~ 60 minutes)*
9. Run 2x Debubble w/ Debubble solution *(~4 minutes)*
10. Run thorough Deep Clean using our Flow Cell Cleaning Solution *(~ 60 minutes)*

    If it is not available: freshly prepare 10% bleach solution (.5% Sodium Hypochlorite)
    https://www.thermofisher.com/order/catalog/product/A43635#/A43635
11. Run performance test (PT)
    - If PT test passes – continue to experiment (Similar to pre-shutdown PT)
    - If PT does not pass, repeat steps 4-7
    - If PT does not pass, follow the troubleshooting guidance on page 5

III. If the System Decontamination Script was successful as part of shutdown

1. Replace focusing fluid filters

   If not already replaced as part of the decontamination protocol performed during long-term shutdown

2. Run 3x Startup *(~10 minutes)*
3. Inspect syringes during startup and replace if necessary:

   Look for dried salt or anything that looks like debris in the syringe barrel and on either side of the plunger; any signs of this would drive syringe replacement.

   - NxT syringe - Cat. No. 100022591
   - Autosampler syringe Cat. No. 4478686
   - CytKick syringe Cat. No. 100054593
4. Run 2x Debubble w/ Debubble solution *(~4 minutes)* (https://www.thermofisher.com/order/catalog/product/A10496#/A10496)
5. Run 2x Rinses *(<1 minute)*
6. Run thorough Deep Clean using Flow Cell Cleaning Solution *(~60 minutes)*

   If it is not available: freshly prepare 10% bleach solution (.5% Sodium Hypochlorite) https://www.thermofisher.com/order/catalog/product/A43635#/A43635

7. Run Performance Test (PT) *(~5 minutes)*
   - If PT test passes – continue to experiment
   - If PT does not pass, repeat steps 2-7
   - If PT passes and values are similar to pre-shutdown continue on with experiments
   - If PT continues to not pass, repeat one more time steps 2-7
   - If PT still does not pass, follow the troubleshooting guidance on page 5

IV. Troubleshooting Guidance

From Attune NxT Flow Cytometer quick maintenance and troubleshooting guide (Pub. No. 100024233)

Observations and Recommended Solutions

1. **Data stream on time plot looks choppy or no events are coming through**
   a. Test for sample syringe movement – Glass syringe located in left side compartment of cytometer.
   b. While observing sample syringe, initiate Rinse function (located on Instrument tab).
      i. 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.
      ii. Perform a power cycle of instrument – Close software and turn off the Attune NxT Flow Cytometer and Autosampler; then turn on the autosampler, then the cytometer, then open software. Log in and perform Startup. While Startup is executing, observe sample syringe.
   c. If syringe is still not moving or appears to be sticking (non-fluid motion), consider changing the syringe before contacting our technical support team.
   d. If sample syringe movement is okay, system may have a clog, consider changing the syringe.
2. **Loose connections errors—system displays an error message (flashing blue backlights) that a fluidics sensor connection is loose (e.g., wash or waste)**
   a. Check to be sure that the bottle is full (focus fluid, wash solution, shutdown) or empty (waste). Fill up all bottles and empty the waste.
   b. Check all fluid lines and sensor connections – Firmly seated and plugged in.
   c. Check the connections for both the cytometer and the autosampler.
   d. If the connections are firm and the error message still shows up, order a new replacement bottle as a new connection is needed. Contact our technical support team.
3. **Leak detection errors**
   a. Leak detection errors—Both the Attune NxT Flow Cytometer and the Autosampler have a leak detector in their bottle bay basins that pick up large leaks (~5 mL):
   b. Inspect to confirm the removal and/or reseating of the fluid bottles when refilled.
   c. 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.

d. 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.
e. Contact our technical support team for assistance. The sensitivity adjustment can be performed while technical support provides instructions.

4. Additional troubleshooting information

a. Attune NxT Flow Cytometer maintenance and troubleshooting guide (Pub. No. 100024234)
b. Attune NxT desktop folder “Attune NxT User Guides”
c. Attune NxT desktop folder “Helpful Attune information” includes training slides PDF, fluorophore guide, consumables list, and quick reference guide

5. Attune NxT Flow Cytometer support contact info

a. For immediate assistance in the US, please call: 1-800-955-6288 (9:00 a.m.–8:00 p.m. ET). Choose option 3 and then option 1 for instrument service and hardware support. Or email instrumentservices@thermofisher.com for the technical assistance center (TAC) and instrument services.
b. **Note:** You will need the serial number of your instrument when contacting our technical support team.
c. If performance tests are failing, please take a photo of the performance test results table, download the log files before calling for assistance.