CytoFLEX SRT
Startup, QC, Cleaning & Shutdown SOP

Daily Startup Procedure & QC: to be followed by first user of the day

Flow Cell Clean Program: to be followed by last user of the day if the CytoFLEX won´t be used in the next 5 days (check the iLab calendar)

Daily Shutdown Procedure: to be followed by last user of the day

Daily Startup Procedure & QC

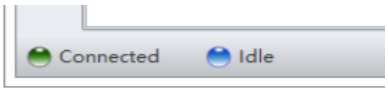
Pre-Startup Inspection

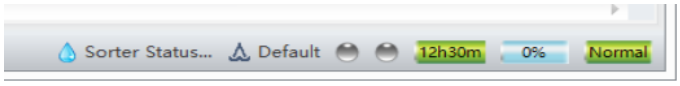
1. If you need to maintain samples and sorted cells at refrigerated temperatures (+4°C) turn on the waterbath *(Note: it will take about 30 minutes to cool the sample chamber and the collection tube/plate holder)*.
2. Inspect the fluidics cart and check that:
    a. The tubing connections for all tanks are correctly placed (Sheath Tank, Waste Tank, Shutdown Solution Tank);
    b. The Sheath Tank is filled to the mark;
    c. The Waste Tank is empty;
    d. The Shutdown Solution Tank contains enough solution (at least half full).
3. Refill Sheath and Shutdown Solution tanks, and empty the Waste tank as needed. *(Note: you must close very tightly the lid on the Sheath tank, otherwise the system will fail to pressurize.)*
4. Startup the biosafety cabinet by pulling up the sash to the mark, and switching on the light and fan.

Daily Startup

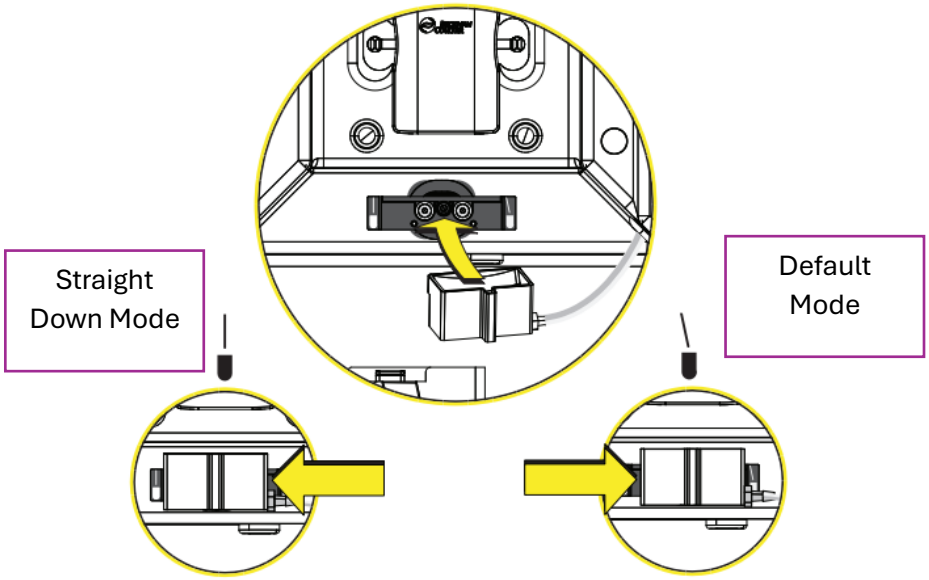
1. Start the computer workstation and log in the **CytExpert** software using the Desktop shortcut

2. Turn ON the sorter by selecting the “Turn On” command in the Cytometer Menu (**Cytometer > Turn On**)
3. Inspect the Status Bar at the bottom of the CytExper window to confirm that:
    a. The workstation is connected to the sorter (“**Connected**”)
    b. The sorter is on “**Idle**” status
    c. The fluidics system is OK (all the 3 tanks indicators are in green)

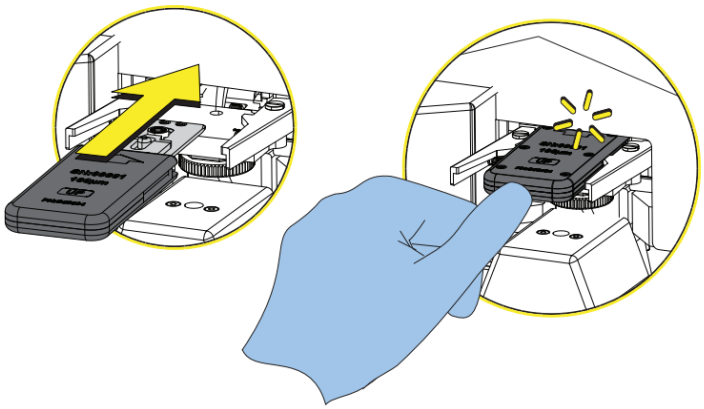




4. Set the Stream Mode:
    a. Select **Sorting > Stream Mode Switch**
    b. Move the Waste Catcher in the **Default Mode** position (for tube sorts) or **Straight Down Mode** position (for plate sorts), then select **Next**, then **Close** in the Stream Mode Switch window. *A Stream Mode mismatch between software and hardware will lead to flooding of the instrument and incorrect sorting.*

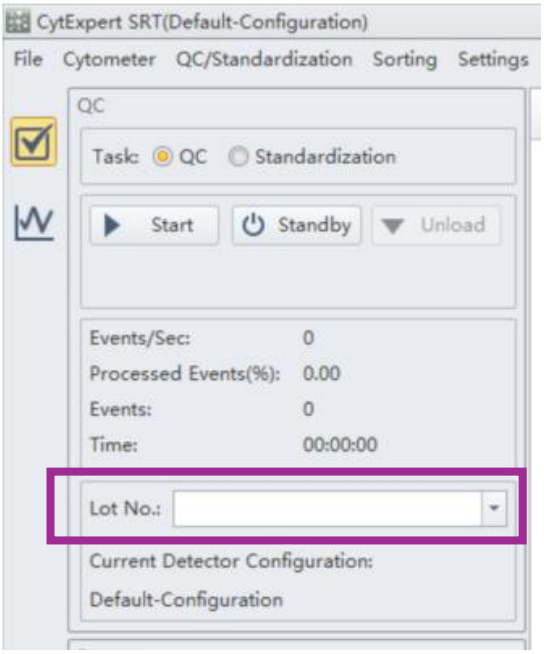


5. Launch the **System Startup Program** from the Cytometer Menu: **Cytometer > System Startup**, and follow the instructions of the wizard.
    a. When prompted, insert the nozzle in the nozzle chamber with the UP symbol facing upwards. The nozzle is locked into position when you hear a click. Close the sort protection door, sliding door and sample station door and press Next.



b. The Startup will proceed with pressurization of the sheath tank, startup of the fluidics & stream and debubbling.
    i. *If the System Startup fails because the sheath tank could not be pressurized, check that the valve in the sheath tank is closed, and re-open & close **tightly** the lid. Repeat the System Startup (leave the nozzle in place).*
c. At the end, the wizard will ask if you want to perform the QC check immediately or not *(Note: QC can be deferred but must be done before the Sort Calibration)*:
    i. Select "Yes" to proceed immediately with the QC check, or
    ii. Select "No", then "Close" to exit the System Startup Program.

Daily QC Check

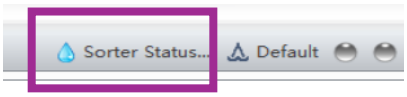


1. Prepare the QC Beads: vortex the vial of **CytoFLEX Ready to Use Daily QC Fluorospheres** and add **5** drops of beads solution to a new FACS tube. *Always store the QC beads vial in the fridge*.
2. In the QC/Standardization Menu, select Start QC/Standardization (**QC/Standardization > Start QC/Standardization**).
3. Select the QC Lot number in the drop-down menu.
4. Insert the FACS tube with the QC beads in the tube holder and press Start to load the sample and start the QC check.
5. Review the QC results:
    a. If QC passes, select "Yes" to start the **Sort Calibration**, or "No" to defer it.
    b. If QC fails, contact the Facility Staff.
6. To retrieve the QC FACS tube:
    a. Select **Unload** in the Acquisition Controls
    b. **Wait** for the tube protection glass cylinder to **fully lift** in the sample chamber
    c. Open the sample chamber door and retrieve the tube.
7. Select Close QC/Standardization (**QC/Standardization > Close QC/Standardization**) to exit the QC Menu.

Sort Calibration (Stream Setup, Side Stream Calibration and Drop Delay determination)

1. In the Sorting Menu, select Sort Calibration (**Sorting > Sort Calibration**).
2. In the Sort Calibration Window:
    a. Check that "**Auto start maintain**" is selected
    b. Do not select "Manual droplet calibration"
3. Make sure that the sort protection door and the sort chamber door are both closed and select **Start** in the Sort Calibration window to start the program.
4. The Program will set up the stream with an optimal breakoff point, optimal side-stream settings and determine the drop delay. At the end of the program (about 6 minutes), close the window by selecting **Close**.

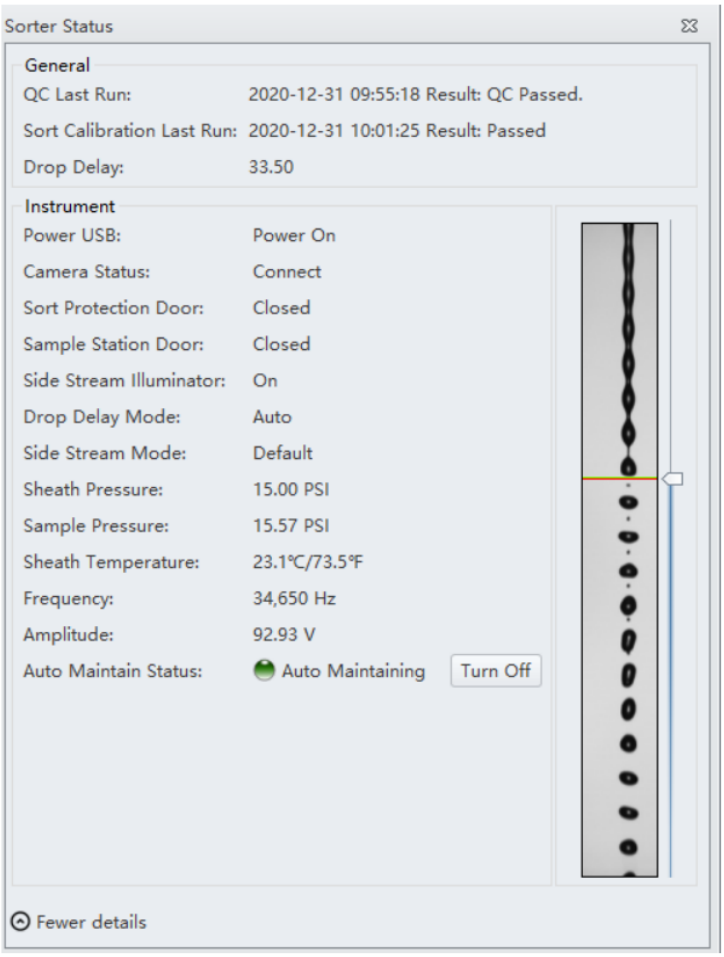
5. You can see the stream live by pressing the **Sorter Status** icon in the Status Bar at the bottom of the CytExpert main window and opening the Sorter Status window.



The Green Line marks the breakoff point set during the Sort Calibration.

Optional: drag the Red Line in correspondence to the Green Line.

Important: Leave the Auto Maintaining Status ON



Cleaning Procedure after every sort

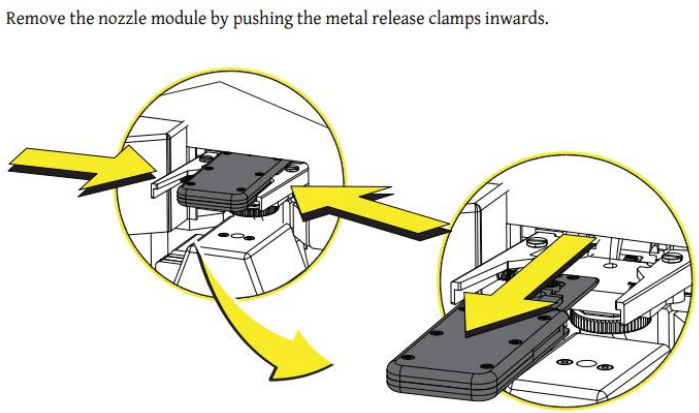
Post-Sort Cleaning (recording is mandatory)

1. Open the **Cleaning Template** on CytExpert (**File > Open Template** or use CytExpert quick menu): the Cleaning Template Experiment is saved in Documents > Templates > 1 – GENERAL).
2. Prepare two cleaning FACS tubes:
    a. 1 tube with 3 mL of FlowClean Solution (Blue solution)
    b. 1 tube with 3 mL of MilliQ Water
3. Load the FlowClean tube in the tube holder, select the FlowClean tube in the Experiment and start recording (Stopping criteria: 5 min at max speed)
4. Load the MilliQ Water tube in the tube holder, select the MilliQ tube in the Experiment and start recording (Stopping criteria: 5 min at max speed)
5. Close the Experiment:
    a. If there are going to be other sorts the same day, select **Standby** to turn off the sheath and log out from CytExpert
    b. If you are the last user of the day, proceed with the **System Shutdown**

Shutdown Procedures

Daily Shutdown

1. Perform the **Daily Clean** Procedure (**Cytometer > Daily Clean**):
2. Prepare two cleaning FACS tubes:
    a. 1 tube with 2 mL of FlowClean Solution (Blue solution)
    b. 1 tube with 3 mL of MilliQ Water
3. When prompted by the wizard, load the FlowClean Solution tube in the tube holder and select Run (3 min clean).
4. When prompted by the wizard, unload the FlowClean Solution tube, load the MilliQ Water tube in the tube holder and select Run (5 min clean).
5. When prompted by the wizard, remove the MilliQ Water tube and select Close.
6. Select **Standby** to turn off the sheath.
7. Check the iLab calendar:
    a. If the CytoFLEX is not going to be used in the next 5 days, do a **Flow Cell Clean Program** before doing the System Shutdown (see next page)
    b. If the CytoFLEX will be used in the next 5 days, go to the next step to perform the shutdown.
8. Select **System Shutdown** (**Cytometer > System Shutdown**)
9. The System Shutdown window appears: follow the prompts.
    a. Remove the nozzle and clean it (see appendix). When done, select **Next**



    b. Select **Yes** to start the System Shutdown (5 minutes).
    c. Remove, clean and re-install the following components (more instruction in the Appendix, page 6):
        i. Nozzle lift
        ii. Side Stream Illuminator
        iii. Deflection Plates
    d. Clean the following components (more instruction in the Appendix; page 6):
        i. Bottom of cuvette
        ii. Sort Chamber
        iii. Side Stream Detector Window
        iv. Collection tube/plate holder
    e. Select Next, and then Close to complete the System Shutdown. The system should be **Connected** but in **Idle** status.
    f. Select **Turn Off** (**Cytometer > Turn Off**) to shut down the sorter.
    g. Exit the CytExpert Software

h. Shutdown the cabinet by switching off light and fan, and pushing down the sash.
i. Turn off the waterbath (if it was in use)
j. Fluidics Cart maintenance:
    i. Refill the Sheath Tank to the mark
    ii. Refill the Shutdown Solution Tank as needed
    iii. Empty the Waste Tank

Flow Cell Clean Program (to be performed before System Shutdown if the sorter won´t be used in the next 5 days)

IMPORTANT *Once you begin, make sure you complete the entire Flow Cell Clean procedure. The entire process takes about 35 minutes.*

1. Prepare a 4ml tube with 50% Contrad (2ml Contrad + 2ml MilliQ water)
2. Select Flow Cell Clean (Cytometer > Flow Cell Clean)
3. In the wizard, **DO NOT SELECT*** "Shutdown after cleaning", and press **Next**.
4. Insert the 50% Contrad tube in the tube holder and press **Next** to start the Flow Cell Clean (about 6 minutes).
5. Remove the 50% Contrad tube and select **Next**.
6. You can skip the cleaning of the following component (Nozzle, Nozzle Lift) as it will be done during the shutdown. Select **Next**.
7. Skip the cleaning of the bottom of the cuvette; select **Next**.
8. Leave the flow cell to soak in Contrad for **30 minutes** (**Step4**). After that incubation time, select Next.
9. Skip **Step5** (Nozzle lift and Nozzle are already inserted). Select **Next**.
10. Select **Close** when the Flow Cell Clean program finishes.
11. Go to **Step 8** of the Daily Shutdown of this SOP and follow the instructions.

** Because of a bug in the software, the "Shutdown after cleaning" in the Flow Cell Clean Program doesn´t work as intended.*
Please always perform first a Flow Cell Clean Program and after its completion, start the Shutdown Procedure.

APPENDIX

Cleaning the nozzle

Fill a clean beaker with DI water and submerge the nozzle in the DI water. The DI water should be clean.

Use an ultrasonic cleaning device to clean the nozzle for 30 seconds.

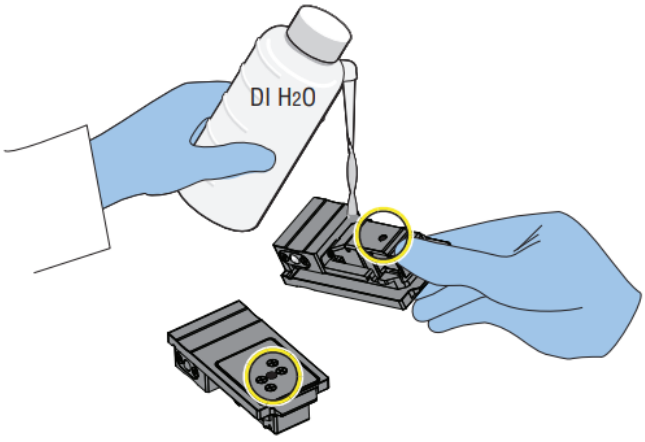
After sonication, dry the nozzle (front and reverse side) using a swab or KIMtech wipe. Do not scratch, press or disturb the O ring to avoid damages.

NEVER USE ETHANOL TO CLEAN THE NOZZLE!!!

Always store the cleaned nozzle in its box.

Cleaning the nozzle lift

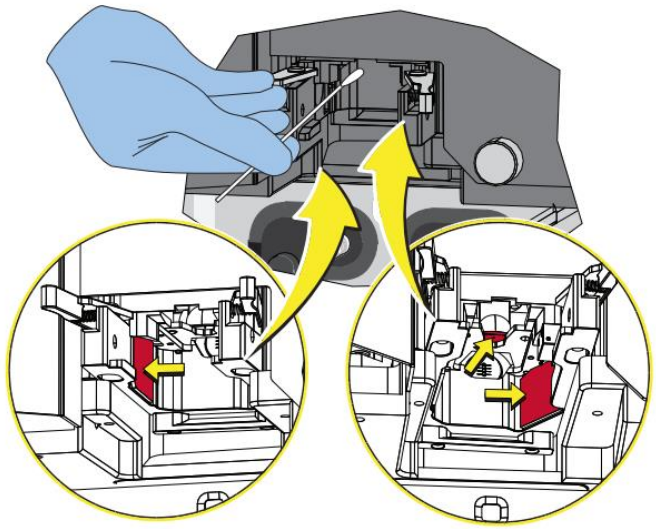
Rinse all surfaces of the nozzle lift with DI water, especially for the central hole where the salt can easily accumulate.



Dry the nozzle lift using a KIMTech wipe.

Cleaning the bottom of the cuvette

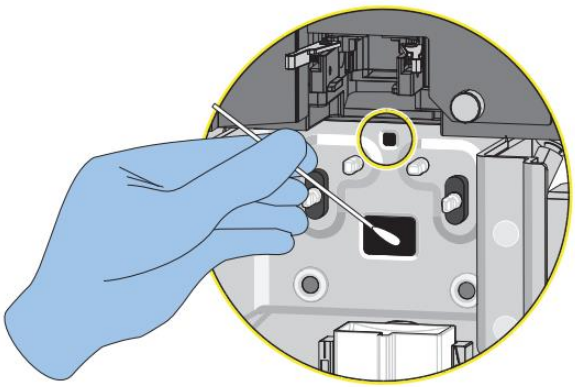
Gently wipe the bottom of the flow cell, and the optics protection glass with the moistened swab.



Use a swab moistened with 70% ethanol

Clean the Side Stream Detection Window

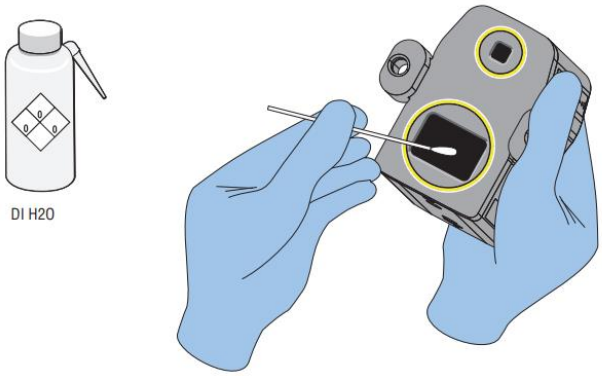
Gently wipe the side stream detection window with the moistened swab.



Use a swab moistened with MilliQ water.

Clean Side Stream Illuminator and Deflection Plates

Gently wipe the side stream illumination source with the swab moistened with DI water.



Gently wipe the deflection plates with the moistened swab.