QUICK GUIDE TO TECHNICAL INSTRUCTIONS FOR USE AND CLEANING OF MD STAINER FOR FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

IMPORTANT

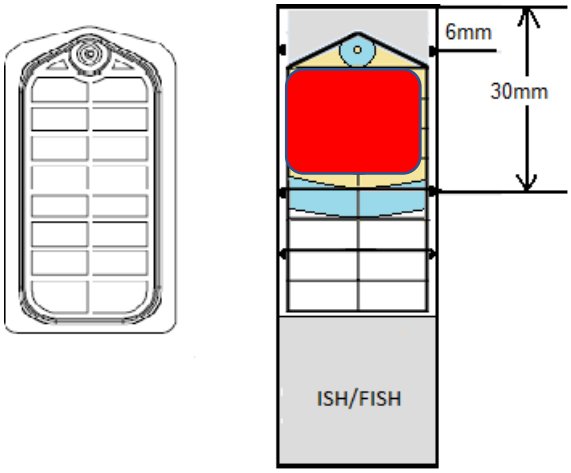
This is a quick guide for the use of MD Stainer and procedure for the fluorescence in situ hybridization technique (FISH), which include the most relevant information for the use, maintenance and cleaning of the instrument, as well as instructions for the preparation of tissue sections. For more detailed information, you can consult the *User Manual*.

For the automated use of the equipment, the user must have been given a previous training by the equipment supplier.

HYBRIDIZATION AREA

In order to guarantee the quality and reliability of the hybridization, the sections must be prepared according to the following guidelines:

- In the picture below, **the obligatory position for the tissue** is marked with a red rectangle.
- The area highlighted in red shows the **hybridization area**. That is why placing the section in the indicated area is important.



PREPARATION AND PRETREATMENT OF PARAFFIN-EMBEDDED SECTIONS

Section

Paraffin-embedded tissue sections are recommended to be **2-3 µm** thick maximum and to place them on the slide respecting the **tissue placement area recommended** in the previous section.

Note: in order to obtain optimal results, it is recommended to prepare the sections a day in advance maximum. If they are made earlier, it is recommended to heat them for a maximum of 5 minutes so that they adhere to the slide correctly and to leave them at room temperature in a protected area until they are to be stained (maximum one week).

HEATING

- The preparations must be **heated right before** introducing them into the MD-Stainer at **60ºC** for **at least 1 hour**. Following these guidelines, the sections will adhere correctly and most of the extra tissue paraffin will be eliminated.
- The preparations may be kept in the heater overnight in case the sections are prepared the previous day to the MD-Stainer start-up.

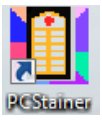
INSTRUCTIONS FOR USE OF THE EQUIPMENT

The MD-Stainer system allows us to work the fluorescence “in situ” hybridization in two ways:

- Fully automated – It includes the whole process, that is: pretreatment of the sample, dispensing of the probes, hybridization and post-hybridization washes.
- Semi-automatic – The first stage is the pretreatment of the sample, followed by the dispensing of the probe by the user manually and a second stage consisting of the denaturation and hybridization of the probe in the instrument. Once the cycle is finished, the user must perform the post-hybridization washes outside the equipment (MAD-FS0105-1 - Post-Hybridization Wash Buffer (Ready to use)).

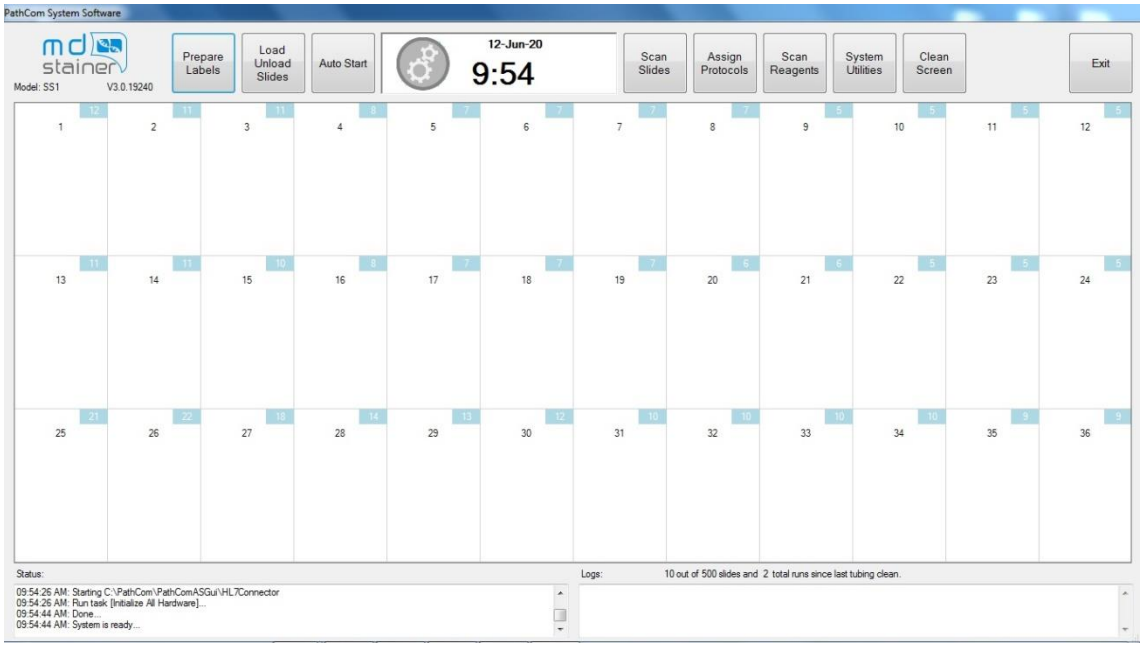
Both forms are described individually in each step of the process of the equipment’s use.

Once the MD STAINER and the computer are on, we must access the following icon to run the software which allows controlling the platform:



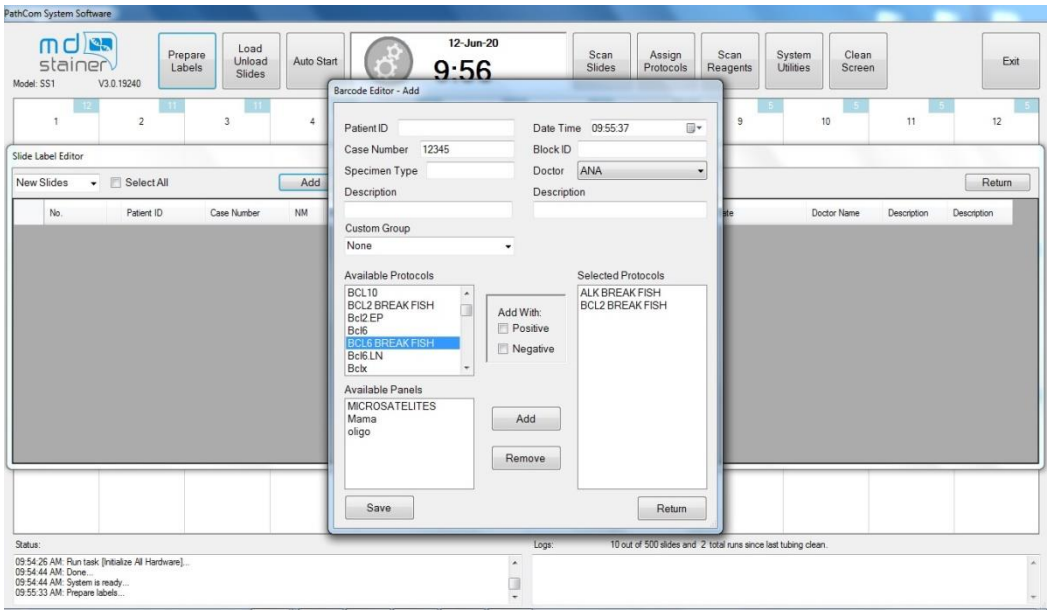
Note: *it is always recommended to turn on* ***the instrument first and the computer afterwards****.*

In the main screen, we have available all the functions to program a FISH cycle we describe in detail below following the daily work order:



1st PREPARE LABELS

In order to prepare the labels, click on **"Prepare Labels"** and select the option create. The fields available will be filled in, which display the information of each sample. Then, assign the techniques and click on *"Save"*. Once all the labels have been created for the necessary techniques, print them and place them in the corresponding preparations.



In **Automatic FISH**, the user must create the labels as described above. All the probes available in automatic FISH end in the word FISH (Example: MYC BREAK FISH).

In **Semi-automatic FISH**, the user does not need to create labels, because he must assign the protocol manually as described below. This is due to the fact that there is not a protocol for each probe, but instead a pretreatment protocol(s) according to the type of processing that each sample requires and that the user requests.

2nd PLACE THE PREPARATIONS IN THE EQUIPMENT

The preparations must be placed in the equipment as the incubation modules are arranged, that is, horizontally, with the label outwards the equipment, by pressing them slightly so that they remain stuck in the support points of each module.

3rd SCAN SLIDES

Its allows the scanning of the preparations previously labelled and placed in the equipment.

- If we choose this option without selecting any of the positions manually, the equipment will scan the 36 positions automatically.
- If the desired positions are previously selected with the left button of the mouse in the slide map (they can be clicked and dragged), the platform will scan the selected positions only.

If you do not have any labels or the determinations to perform do not require them, the system allows the placing of the preparations and the assignment of the respective protocols **manually** by means of the option ***"Assign Protocols".*** To do this, select one position of the corresponding slide (or more than one) with the left button of the mouse, click on **"Assign Protocols"** and a

new window will open with all the techniques available from which you can select the desired technique.

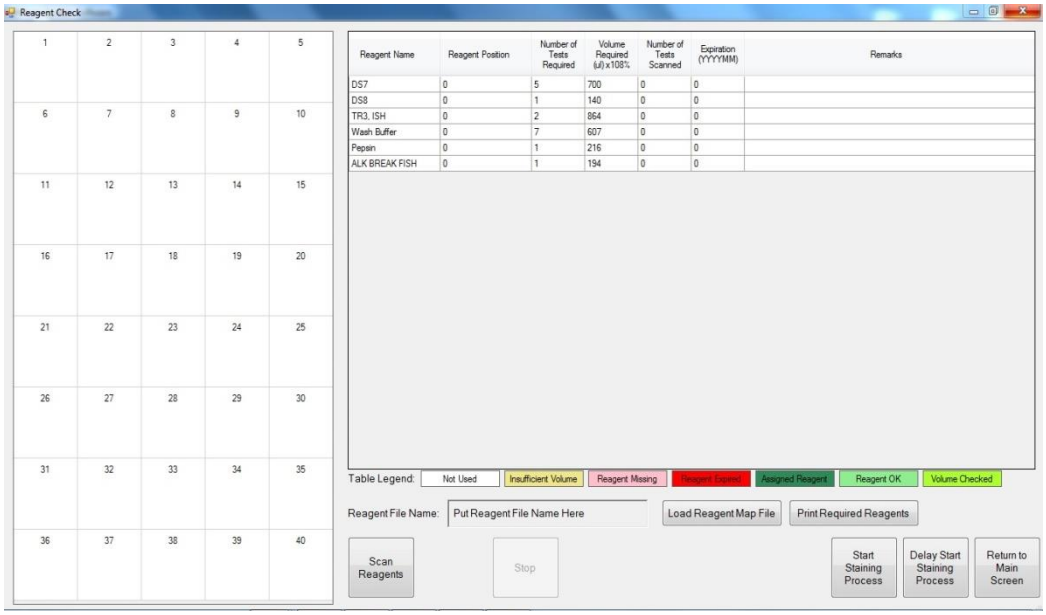
In **Automatic FISH**, you must select the option scan labels to read the labels printed and places on the slide.

In **Semiautomatic FISH**, for which no labels were created, you must select the option "Assign protocols" to assign the pretreatment protocol you wish to use according to the type of sample. The standard protocol by default is named FISH PRETRATAMIENTO.

Once the Pretreatment of the samples is finished, the user must dispense manually the probe(s), place the cover slip, seal with - Rubber Cement Fixogum (Reference: MAD-011530Q-125) and reload them in the instrument to perform the- denaturation and hybridization. To do this, the user must use the option "Assign protocols" again to assign the hybridization protocol you wish to use according to the type of probe. The standard protocol for the probes supplied by Vitro SA is named FISH HIBRIDACIÓN by default.

4th SCAN REAGENTS

Once the techniques to perform have been scanned and assigned manually, the user must Scan Reagents. When clicking on this option, a screen will appear, displaying all the necessary reagents to complete the process, as well as the volume and number of tests necessary for each one.



Once the reagents have been placed in the rack, click on ***"Scan Reagents"*** and the scanning process will start automatically.

The reagents scanned will be shown in their corresponding position in the rack, in the reagents map on the left (positions 1-40) and it will display the following information:

- Reagent name.
- Current number of tests.
- Expiration date.
- RFID labels identification.

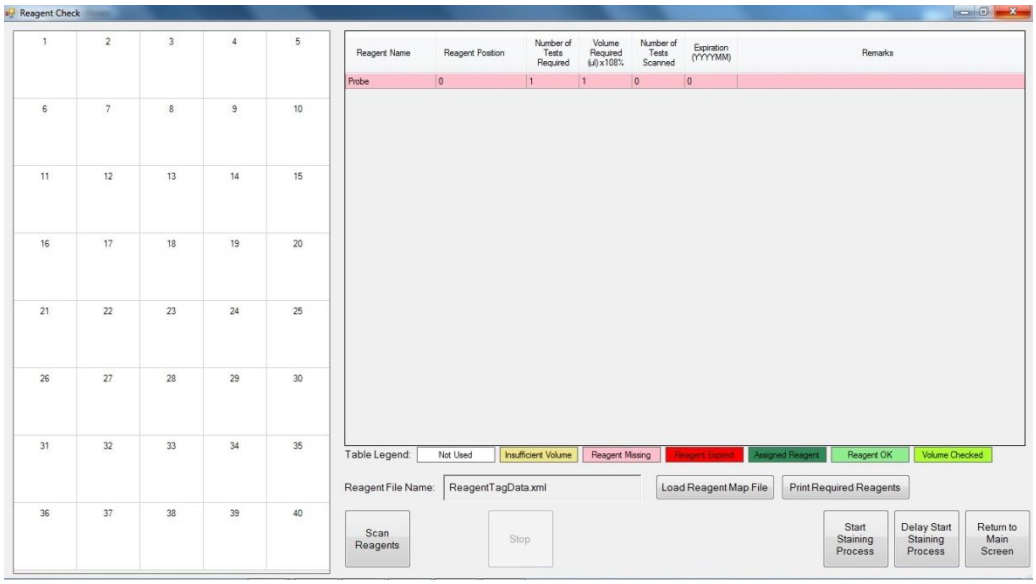
During this process, MD-Stainer can identify and inform about the following possible issues during programming by means of different colors:

White - Not used. There is no techniques that need this reagent.
Yellow - Insufficient. Insufficient number of tests.
Pink - Missing reagent. The system did not detect this reagent in the rack.
Red – Expired. It expired.

In any case, the technician must **replace the expired reagents, place the missing ones or with insufficient number of tests** and repeat the scanning process.

Once all the scanned reagents are shown in green, everything is ready to start the process.

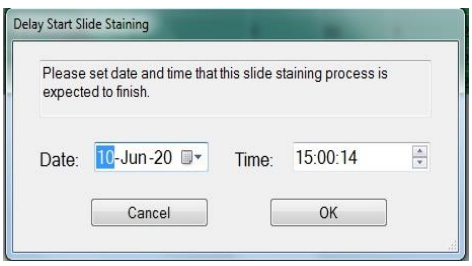
Both for automatic and semiautomatic FISH, the user must proceed with all the scanning process of reagents as described above. The only difference between both processes (Automatic and Semiautomatic) is the requirement of placing the ready-to-use probe in the reagents rack for the automatic FISH, since for the Semiautomatic FISH, the probe is dispensed manually by the user, being the user who must only assign manually and fictitiously in the reagent "**Probe**" to perform the hybridization protocol.



5th START STAINING PROCESS

There are two options to start the process:

1. ***Start staining process***. In this case, the process will start immediately.
2. ***Delay Start***. It allows delay the start. The user must enter the date and time the process must end in the system. (important to consider the values AM/PM).



Note : *For hybridization cycles only, you must not use the option of delay Start to avoid that the probe remains on the tissue without the appropriate temperatures for the hybridization process, since, if the hybridization process does not start right away, the preparation will remain at room temperature up to the start time. In this case, you must start the hybridization process immediately.*

For the Pretreatment of the samples in the Semiautomatic or fully Automated FISH, you must use the delay start to perform the process overnight.

When the process starts, the user must make sure that both **the wash buffer and the waste capacity are sufficient**. To do this, the system displays a screen with the necessary total volume for both and once checked, the user must press **OK** to start the process.

6th FINISH STAINING PROCESS

The system will mark in light green the slides that completed the staining successfully and will name them as "**Finished"**. After completing the last step of the process, the systems unlock the MD-Stainer's door and will generate a notification with alarm for the user: **"Process successfully Ended"**.

Once the whole staining process has finished, the modules used will remain closed, keeping all the preparations hydrated. In order to take the preparations from the instrument, press "Ok" in the notification **"Process successfully Ended"** and press ***Load / Unload Slides*** *to open the modules*.

Once the process of **automatic FISH** is finished, the user must remove the preparations and place them in a cuvette with distilled water and wash them for 30 seconds away from light. Later, let the preparations dry in the dark and mount them with fluorescent contrast medium, Fluorescent Contrast Agent - DAPI+AntiFade (Reference: MAD-FS0106-1).

Once the **Semiautomatic FISH** process is finished, the user must remove the preparations and place them in a cuvette with distilled water and continue with the post-hybridization washing process recommended, Post-Hybridization Wash Buffer (Ready to use) (Reference: MAD-FS0105-1). Later, let the preparations dry in the dark and mount them with fluorescent contrast medium, Fluorescent Contrast Agent - DAPI+AntiFade (Reference: MAD-FS0106-1).

OTHER OPTIONS AVAILABLE (Not necessary in the routine use of the equipment)

Load / Unload Slides

This function is used to open and close the incubation modules and thus allows the load or unload of slides at the beginning and end of the staining process. Nonetheless, once the system is started, all the modules remain open automatically so that the user can introduce the slides without having to select this option.

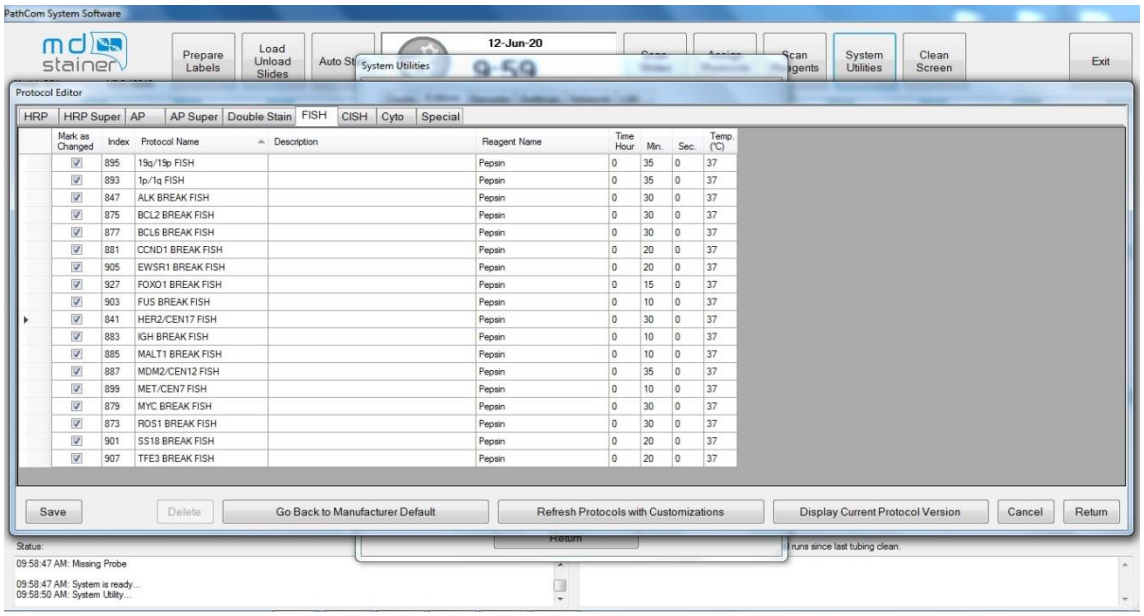
Automatic start

To start a preset program.

System

This section describes all the configuration options to control, check the system, create and modify reagents and protocols. For more detailed information on this section, please consult the User Manual.

There is a function we must mention in this quick guide. It is the one which allows the user to adjust the Pepsin time, that is, the digestion time for each of the probes available in automatic format. To access this option, you must click on System – Editors – Protocol Editor – FISH and adjust the pepsin time for each probe according to the needs.



For the Semiautomatic FISH, we offer a standard pretreatment protocol of the samples. In case you need additional protocols with different functioning conditions, contact your equipment's supplier.

Clean screen

It allows cleaning the screen once the process has finished or when the user assigns a protocol by mistake.

Exit

It allows exiting the software.

CLEANING AND PREVENTIVE MAINTENANCE

MANDATORY CLEANING PROTOCOL

It is necessary to clean the MD-Stainer on a recommended timely basis to maintain the reliability, shelf life of the system and the staining quality.

1. CLEANING OF THE MODULES AND HOT PLATES (DAILY)

The modules must be cleaned every day to remove any reagent remains and avoid any accumulation in the instrument.

- Unload all the slides from the system.
- **It is recommended to use blotting paper,** smooth, that cleans the excess that remains after the staining cycle both on the modules and on their surroundings.

2. CLEANING AND INSPECTION OF THE INCUBATION CHAMBERS (DAILY)

In order to avoid the accumulation of salts and reagent waste during the staining cycle, the chambers must be cleaned every day. During the cleaning, you must look for any cracks, leaks or degradation on the chamber surface.

To do this, after every staining cycle, clean the surface of the chambers with an alcohol swabs (Reference: MAD-MDSCW - Cleaning wipes for MD-Stainer). It is not necessary to remove the chambers from the instrument.

If, during the cleaning, you spot any anomalies on the chamber such as cracks and/or degradation when pressing the chamber, you must replace it with a new incubation chamber (Reference: MAD-CH36 - Incubation Chambers for MD-Stainer).

3. THOROUGH CLEAN OF THE CHAMBERS

If you only use the MD-Stainer to perform FISH (automatic or semiautomatic), there is not a specific periodicity for the thorough cleaning of the chambers, since none of the reagents used make them dirty. Therefore, the correct execution of the cleaning and daily inspection of the chambers is enough to keep them in a good state up to the end of their shelf life.

The cleaning is **obligatory** indeed on a weekly basis as long as that the system itself combines immunohistochemistry techniques, CISH and FISH. Refer to the user manual or the quick guides for Immunohistochemistry and/or CISH for a correct cleaning.

4. REPLACEMENT OF ALL THE INCUBATION CHAMBERS

For the FISH technique, the incubation chambers have an estimated shelf life according to the frequency and number of preparations performed in each FISH cycle. Thus, as an orientation, for about 1500 preparation/year, it is estimated that the replacement of chambers would be necessary every three months, as long as the considerations described above are taken.

For clients with a lower work volume, the replacement of all the chamber can be extended as long as no anomalies are detected.

5. CLEANING OF THE UPPER SURFACE OF THE WASHING STATIONS AND THE PROBES Z1/Z2 (WEEKLY)

Salt and reagent remains can also accumulate on the upper surface of the washing stations and the probes Z1/Z2 with the prolonged use of the instrument. Clean the waste with a cotton/towel moistened in alcohol.

6. VISUAL INSPECTION OF THE MODULES, ROBOT AND VERIFICATION OF THE CORRECT PERFORMANCE (WEEKLY)

Contact your MD-Stainer supplier for any anomalies detected or supply of spare parts and consumables.

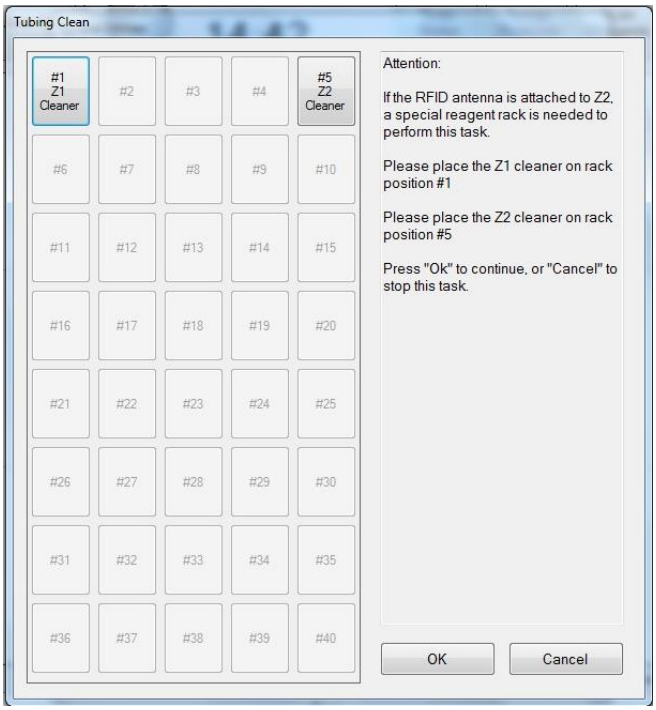
7. CLEANING OF SUCTION/DISPENSING TUBES (FORTNIGHTLY OR EVERY 400 DETERMINATIONS)

The tube corresponding to Z2 can accumulate waste of some reagents with the prolonged use of the instrument. It is recommended to inspect regularly and visually both tubes Z1 and Z2 and perform the cleaning if necessary.
The system itself also tracks the number of determinations performed before the cleaning cycle. By default, 400 determinations are programed once this figure is reached. The system will then automatically request the tube cleaning.

To do this:

- Load the reagent rack, especially with the cleaning solutions for Z1 and Z2 (Reference: MAD-TCLK- Tubing Cleaning kit).
  - Place the rack in the system.
  - Connect the fluid system (Washing buffer).
  - Access to “System” in the main screen, and click on “Tube Cleaning”.
  - Follow the instructions below:
    a) Place the cleaning solution Z1 in the # 1 position of the reagent rack as shown in the image.
    b) Place the cleaning solution Z2 in the # 5 position of the reagent rack as shown in the image.

c) Click on “OK” to start the tube cleaning.



Each probe, Z1 and Z2 aspirates 5 ml of cleaning solution from their corresponding vials placed in their respective position of the reagent rack. Then, the system will show a 20-minute countdown.

After 20 minutes, the instrument will initialize the system automatically and will purge the waste of the cleaning solutions. Click on “Cancel” anytime to purge the tube waste immediately.

Preventive maintenance by the Technical Service

The preventive maintenance will be carried out for the whole system by a trained technician. Contact your MD-Stainer supplier to program the maintenance.