Pu·MA System® 3D

How to use the Pu·MA Protocol Template

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General Introduction

The Pu·MA System performs assays using a flowchip that automates all the incubation, wash, substrate steps, and media exchanges. Reagents are loaded into the flowchip, the flowchips placed into the Pu·MA System benchtop instrument, the assay program is accessed on the touchscreen and then press Run. At the end of the protocol, the flowchips are removed and ready for analysis.

All Pu·MA System assays are executed as sequences of fluid transfers. For example, the entire 20 µL volume of fluid is transferred from a reagent well to a sample well, followed by incubation time for the defined period of time, and then transfer of the fluid back from the sample well back to the reagent well, where the collected supernatant is stored till the end of the Pu·MA protocol. This supernatant can be used for the downstream analysis of secreted factors for downstream applications. **Fig A** shows a schematic of a short sequence of fluid transfers.

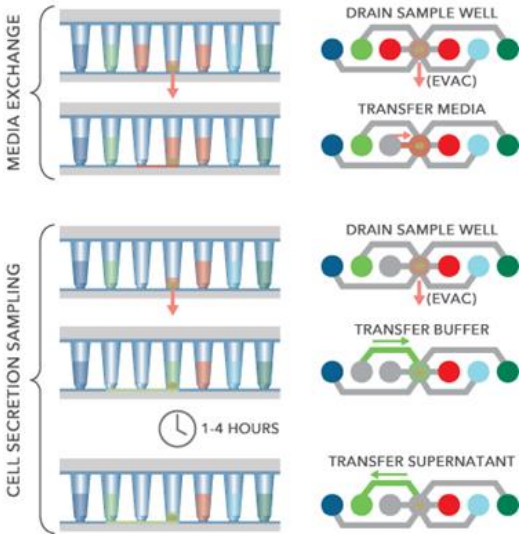


Fig A. Schematic fluid transfers within Pu·MA System flowchip

Anatomy of the Flowchip

Each Pu·MA System flowchip contains eight lanes of reagent wells connected by microfluidic channels (**Fig B**).

<u>Key features of the flowchip</u>:

- Each reagent well is connected to the sample well (#6) via separate channels, that are isolated from other channels preventing fluid cross-contamination.
- Reagent wells are not connected to each other.
- Flowchip can be filled with any reagents (media, compounds, stains, etc.) depending on the assay set up.
- Organoids/spheroids are loaded into the sample well and located in a protected chamber at the bottom of the well.
- The diameter of the protected sample chamber is 1.2 mm and can accommodate samples up to 1mm in size.
- Reagent can be directed in and out of the sample well without disturbing or drying out the sample.
- The bottom of the flowchip is a thin cyclic olefin copolymer (COC) film which makes it compatible with high resolution imaging.

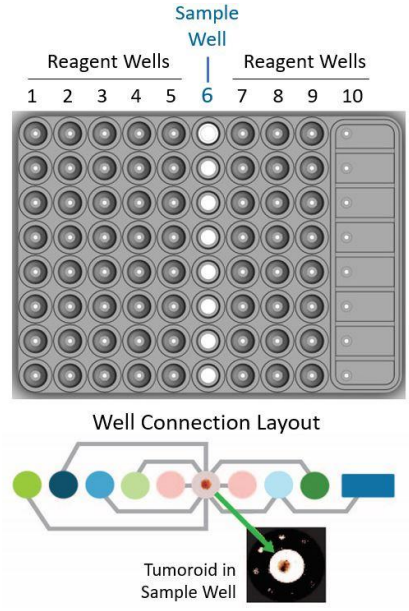


Fig B. Schematic of Pu·MA System flowchip and well connections (single flowchip)

Flowchip and Holder Orientation

- Remove 3D Flowchips from bag and place 4 flowchips into the holder as shown in Fig B.
- Make sure the indexing notch of the holder is facing the top left part of the system (A1 position **Fig C**).
- This is the correct orientation to place the flowchip holder into the Pu·MA system as well.

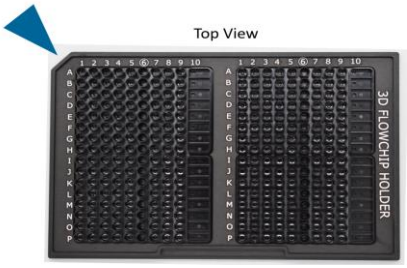


Fig C. Flowchip holder notch facing top left hand-side in correct orientation for loading into system (4 flowchips in the holder)

Precautions for Assay Prep

- Wear gloves while performing the assay.
- Place flowchips into a flowchip holder in the correct orientation (as shown in Fig B).
- If you are using less than 4 flowchips the unused positions must be filled with sealed blanking flowchips.
- **To load reagent wells**: use good Pu·MA System pipetting technique as shown **Fig D**. The pipette tip **should be inserted vertically** in the center of each well. The liquid should be dispensed from near the bottom of the well (~1mm from bottom).
- **DO NOT GO TO SECOND STOP OF PIPETTE.**
- **To load spheroid/organoids sample:** use our standard loading guidelines (**Fig E**).
    - Prime the tip with 1% BSA, then aspirate organoid from the microplate along with 4 µL of media.
    - Let the organoid sediment in the tip and carefully dispense it to the center of Well 6 in 2 µL (**Fig E1**).
    - Dispense 20 µLof media to Well 6 as shown in **Fig E2**.

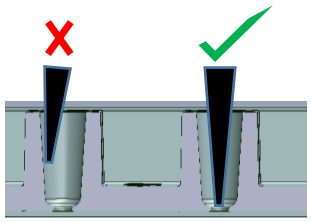


Fig D. Good pipetting method for Pu·MA System

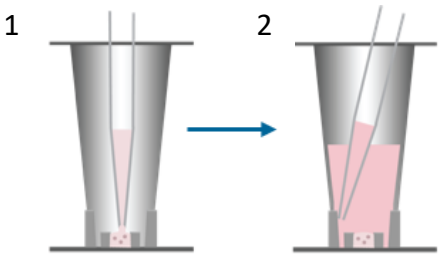


Fig E. Loading organoids and media into Well 6.

1) How to use the Pu·MA protocol template

Open Pu·MA System Software and select “Connect.” There are two folders under the “Device folder” drop down that protocols can be stored in: Standard or Custom. Protocols in the “Standard folder” are provided by Protein Fluidics and will not be able to be deleted or overwritten. They are intended to be used as a reference to build custom protocol files from. “Assay Protocol” drop down lists protocols stored in the Pu·MA System.

A) Template

To start working with the template go to STANDARD folder. From a pull down menu of Assay Protocol select “Transfers Template 102022” protocol (as shown in **Fig F**).

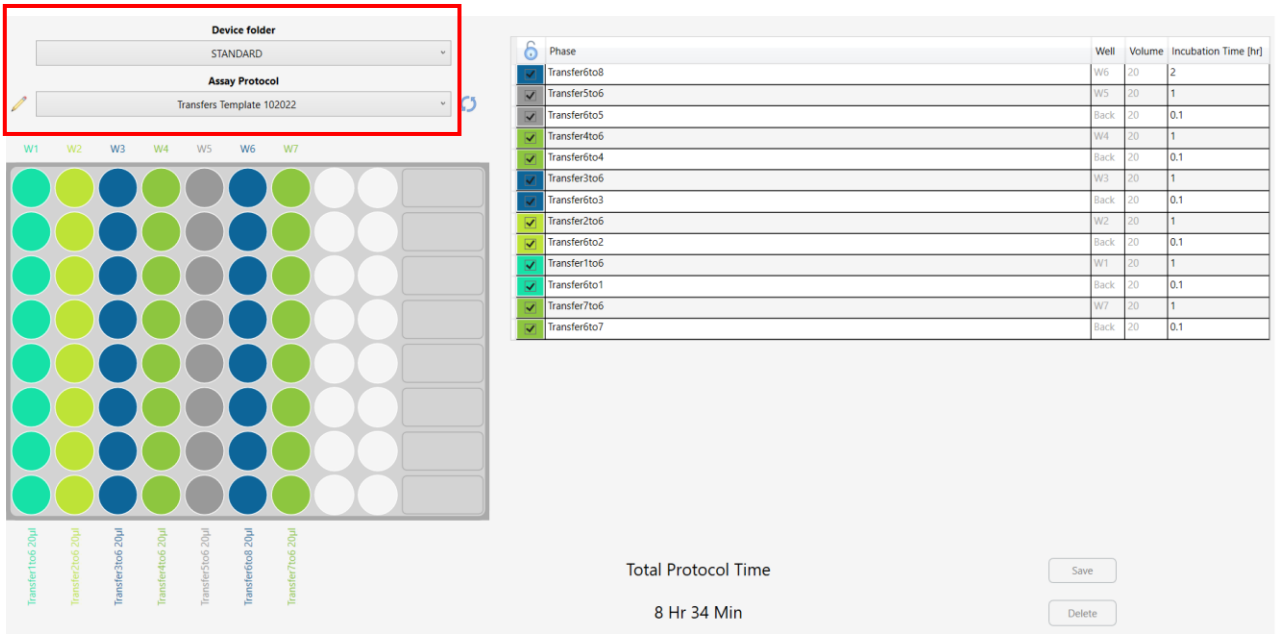


Fig F. Screenshot from Pu·MA software.

B) Phase Column

- Each line in Phases column shows which fluid transfer is executed at each step of the protocol (**Fig G**). For example the first phase line indicates transfer from well 6 to well 8. The second line shows the transfer step from reagent well 5 to sample well 6. Line 3 shows that the fluid will transferred back from the sample well 6 to well 5.
- The name of each phase can be changed if needed. For that ouble click to highlight the phase name and modify. After that press ENTER and move to the next phase.
- If your protocol does not require all of the steps, simply uncheck the phases/transfers that you don't need and they will not be executed during the protocol. To check/uncheck phases first unlock the "lock" icon and proceed with the protocol modification.

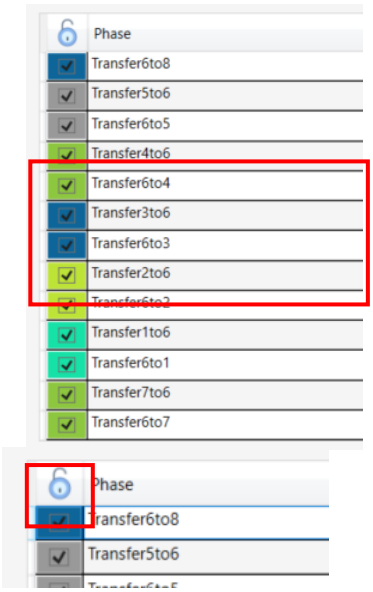


Fig G. Phase Column

C) Time Column

- Each line in Time Column indicates for how long the sample will be incubated in well 6. Note that the time is set in hours.
- The incubation time can never be set to 0 because it accounts for the time that is required for the fluid transfer to be executed. Usually it takes few minutes to complete fluid transfer. Therefore the minimal incubation time can be set to 0.1 hours.
- All "back" transferres (red boxes) are set by default to 0.1 hour. If you run the protocol without stops, there is no need to modify it. The possibility to change incubation time in "back" phases adds flexibility in the situation when the protocol has to be stopped in the middle and then resumed (see examples below).
- **Example:** The incubation time in line 2 is set to 3 hours. That means that the sample will be incubated for 3 hours with the reagent that was transferred from well 5 to sample well 6. (**Fig H**)

Phase	Well	Volume	Incubation Time [hr]
Transfer6to8	W6	20	1
Transfer5to6	W5	20	3
Transfer6to5	Back	20	0.1
Transfer4to6	W4	20	3
Transfer6to4	Back	20	0.1
Transfer3to6	W3	20	3
Transfer6to3	Back	20	0.1
Transfer2to6	W2	20	3
Transfer6to2	Back	20	0.1
Transfer1to6	W1	20	3
Transfer6to1	Back	20	0.1
Transfer7to6	W7	20	3
Transfer6to7	Back	20	0.1

Fig H. Time Column

D) Flowchip Image Outline

- The flowchip outline image (**Fig I**) shows the wells that should be loaded with the reagents and well 6 will contain the organoid/spheroid sample.
- The template shows a protocol with wells 1 through 7 filled with sample and reagents.

NOTE: Wells 8 through 10 are not colored indicating that they <u>should not</u> be loaded with reagents.

Fig I. Flowchip Image Outline

2) How to save modified protocols

- The modified template protocol can be saved under a new name. For that click on "Save" icon and type the new name (shown below **Fig J**).

Fig J. Pu·MA software icons

- The protocol automatically will be saved to the CUSTOM folder.
- The original template will remain in STANDARD folder. It is locked for any modifications and cannot be overwritten.
- You can further modify your newly created protocol in the CUSTOM folder (**Fig K**).
- To save your modification you can click green SAVE button if you would like to overwright the existing protocol. Alternatively you can save it under a new name using "Save" icon (**Fig L**).

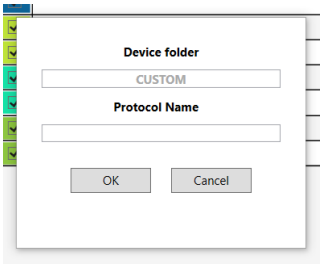


Fig K. CUSTOM folder

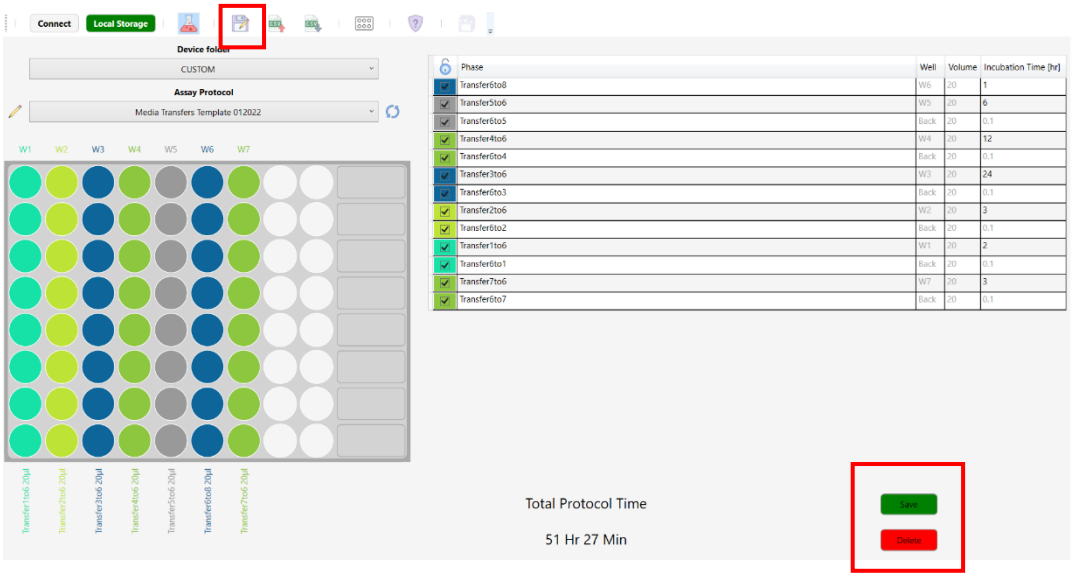


Fig L. Save icon & Save button in Pu·MA software

3) Protocol Steps

A) When all steps are selected

Below are the steps that are automatically executed by the Pu·MA System if ALL the steps are checked.

The template is designed to start any assay protocol with the pre-equilibration incubation step before the beginning of fluid transfers. We recommend to set it to at least 0.5 hours to allow sample and reagents to pre-equilibrate after loading and before your assay starts. Here is the sequence of steps which will be executed (**Fig M**).

- Pre-equilibrate for 1 hour > remove loading fluid/media from sample in well 6: Transfer 6 → 8
- Transfer reagent from well 5 to sample well 6: Transfer 5 →6 > Incubation
- Remove reagent from sample well 6 back to well 5 (collected supernatant): Transfer 6 →5
- Transfer reagent from well 4 to sample well 6: Transfer 4 →6 > Incubation
- Remove reagent from sample well 6 back to well 4 (collected supernatant): Transfer 6 →4
- Transfer reagent from well 3 to sample well 6: Transfer 3 →6 > Incubation
- Remove reagent from sample well 6 back to well 3 (collected supernatant): Transfer 6 →3
- Transfer reagent from well 2 to sample well 6: Transfer 2 →6 > Incubation
- Remove reagent from sample well 6 back to well 2 (collected supernatant): Transfer 6 →2
- Transfer reagent from well 1 to sample well 6: Transfer 1 →6 > Incubation
- Remove reagent from sample well 6 back to well 1 (collected supernatant): Transfer 6 →1
- Transfer reagent from well 7 to sample well 6: Transfer 7 →6 > Incubation
- Remove reagent from sample well 6 back to well 7 (collected supernatant): Transfer 6 →7

Phase	Well	Volume	Incubation Time [hr]
Transfer6to8	W6	20	1
Transfer5to6	W5	20	1
Transfer6to5	Back	20	0.1
Transfer4to6	W4	20	1
Transfer6to4	Back	20	0.1
Transfer3to6	W3	20	1
Transfer6to3	Back	20	0.1
Transfer2to6	W2	20	1
Transfer6to2	Back	20	0.1
Transfer1to6	W1	20	1
Transfer6to1	Back	20	0.1
Transfer7to6	W7	20	1
Transfer6to7	Back	20	0.1

Fig M. Screenshot when all protocol steps are selected

B) When all steps are not selected or required

If you don't need to execute all the steps from the template, you can stop after any phase by unchecking all the steps below that point. Note that if you stop the protocol after the step when reagent is transferred **to** your sample in well 6, that sample will be in 20 µL of volume.
If you stop your protocol after the reagent is transferred back **from** well 6 to its original well, your sample will be left almost without any fluid and can dry if left unattended for long. For example, lets say you would like to execute transfers using only reagent wells 4 and 5. You can set your protocol using two options as shown below:

Option 1: The last executed step would be Transfer 4 →6 > Incubation. All steps below in the protocol set

	Phase	Well	Volume	Incubation Time [hr]
☑	Transfer6to8	W6	20	1
☑	Transfer5to6	W5	20	1
☑	Transfer6to5	Back	20	0.1
☑	Transfer4to6	W4	20	1
☐	Transfer6to4	Back	20	0
☐	Transfer3to6	W3	20	0
☐	Transfer6to3	Back	20	0
☐	Transfer2to6	W2	20	0
☐	Transfer6to2	Back	20	0
☐	Transfer1to6	W1	20	0
☐	Transfer6to1	Back	20	0
☐	Transfer7to6	W7	20	0
☐	Transfer6to7	Back	20	0

Fig N. Screenshot for Option 1, when last step will be Transfer 4 →6 > Incubation

up should be unchecked. **At the end your sample will be in 20 µL of fluid** (**Fig. N**).

	Phase	Well	Volume	Incubation Time [hr]
☑	Transfer6to8	W6	20	1
☑	Transfer5to6	W5	20	1
☑	Transfer6to5	Back	20	0.1
☑	Transfer4to6	W4	20	1
☑	Transfer6to4	Back	20	0.1
☐	Transfer3to6	W3	20	0
☐	Transfer6to3	Back	20	0
☐	Transfer2to6	W2	20	0
☐	Transfer6to2	Back	20	0
☐	Transfer1to6	W1	20	0
☐	Transfer6to1	Back	20	0
☐	Transfer7to6	W7	20	0
☐	Transfer6to7	Back	20	0

Fig O. Screenshot for Option 2, when last step will be Transfer 6 →4

Option 2: The last executed step would be Transfer 6 →4. All steps below in the protocol set up should be unchecked. **At the end your sample will be without any fluid** (**Fig. O**).

4) How to stop and resume a protocol during an assay

The protocols **CANNOT BE STOPPED AND RESUMED** in the middle of a run. If you **need to make a stop**, for example to examine the flowchip and then to continue with the assay + fluid transfers, you will need to **create two separate protocols**.

For example, let us say you would like to execute transfers using reagents from wells 4 and 5, then stop the protocol, and then resume fluid transfers. To do this, you will need to create two protocols.

1st Protocol : The first protocol will execute fluid transfer steps as follows (**Fig P**):

- Pre-equilibrate for 1 hour > remove old fluid/media from sample in well 6: Transfer 6 → 8
- Transfer reagent from well 5 to sample well 6: Transfer 5 →6 > Incubation
- Remove reagent from sample well 6 back to well 5 (collected supernatant): Transfer 6 →5
- Transfer reagent from well 4 to sample well 6: Transfer 4 →6 > Incubation > STOP

	Phase	Well	Volume	Incubation Time [hr]
☑	Transfer6to8	W6	20	1
☑	Transfer5to6	W5	20	1
☑	Transfer6to5	Back	20	0.1
☑	Transfer4to6	W4	20	1
☐	Transfer6to4	Back	20	0
☐	Transfer3to6	W3	20	0
☐	Transfer6to3	Back	20	0
☐	Transfer2to6	W2	20	0
☐	Transfer6to2	Back	20	0
☐	Transfer1to6	W1	20	0
☐	Transfer6to1	Back	20	0
☐	Transfer7to6	W7	20	0
☐	Transfer6to7	Back	20	0

Fig P. Screenshot for 1st protocol, when last step will be Transfer 4 →6 > Incubation

2nd Protocol: This second protocol will start with completion of a Transfer 6 →4. All the steps above this point in the template should be unchecked (**Fig Q**).

- Incubated you sample if needed (incubation is set to 6h incubation in this example)
- Remove reagent from sample well 6 back to well 4 (collected supernatant): Transfer 6 →4
- Transfer reagent from well 3 to sample well 6: Transfer 3 →6 > Incubation
- Remove reagent from sample well 6 back to well 3 (collected supernatant): Transfer 6 →3
- Transfer reagent from well 2 to sample well 6: Transfer 2 →6 > Incubation
- Remove reagent from sample well 6 back to well 2 (collected supernatant): Transfer 6 →2
- Transfer reagent from well 1 to sample well 6: Transfer 1 →6 > Incubation
- Remove reagent from sample well 6 back to well 1 (collected supernatant): Transfer 6 →1
- Transfer reagent from well 7 to sample well 6: Transfer 7 →6 > Incubation
- Remove reagent from sample well 6 back to well 7 (collected supernatant): Transfer 6 →7

Note that there is an option to set your sample for an incubation (6h in this example) if needed before continuing 2nd transfer protocol. This incubation step can be incorporated in any "back" transfer phase depending on where your previous 1st protocol was stopped.

Phase	Well	Volume	Incubation Time [hr]
Transfer6to8	W6	20	0
Transfer5to6	W5	20	0
Transfer6to5	Back	20	0
Transfer4to6	W4	20	0
Transfer6to4	Back	20	6
Transfer3to6	W3	20	1
Transfer6to3	Back	20	0.1
Transfer2to6	W2	20	1
Transfer6to2	Back	20	0.1
Transfer1to6	W1	20	1
Transfer6to1	Back	20	0.1
Transfer7to6	W7	20	1
Transfer6to7	Back	20	0.1

Fig Q. Screenshot for 2nd protocol, when last step will be Transfer 6 →7

5) Checking / Unchecking Unused Transfers

You can modify the protocol by checking/unchecking Phases. We recommend to eliminate unused phases either from the top or from the bottom of the template sequentially. Avoid skipping unused phases in the middle of the protocol as shown in **Fig R**.

AVOID

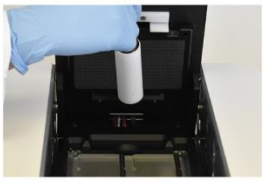
Phase	Well	Volume	Incubation Time [hr]
Transfer6to8	W6	20	1
Transfer5to6	W5	20	1
Transfer6to5	Back	20	0.1
Transfer4to6	W4	20	1
Transfer6to4	Back	20	0
Transfer3to6	W3	20	0
Transfer6to3	Back	20	0
Transfer2to6	W2	20	0
Transfer6to2	Back	20	0.1
Transfer1to6	W1	20	1
Transfer6to1	Back	20	0.1
Transfer7to6	W7	20	1
Transfer6to7	Back	20	0.1

Fig R. Screenshot of phase selection that should be avoided to prevent protocol failure

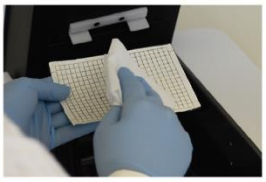
6) Recommended Best Pu·MA System Maintenance Practices

Before Assay

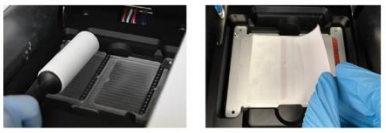
Clean top gaskets with a lint roller & compressed air to remove debris. Wipe using 70% ethanol or IPA on a Kimwipe.



Clean membrane with a lint roller & to remove debris. Wipe using 70% ethanol or IPA on a Kimwipe.

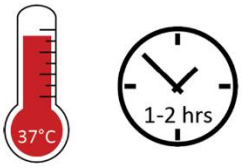


Clean baseplate gaskets with a lint roller & compressed air to remove debris. Wipe using 70% ethanol or IPA on a Kimwipe.

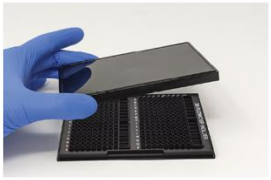


Assay Prep

Bring media & reagents to 37°C for 1-2 hrs, or vacuum degas, before loading into flowchips.



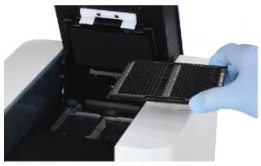
Keep flowchips covered when not dispensing to prevent debris & contamination.



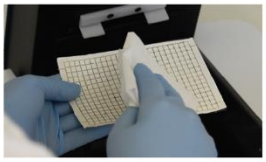
Do not exceed 1% BSA concentration for dispensing into flowchips.

After Assay

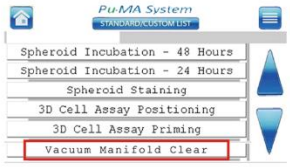
Remove the flowchip holder with flowchips from the Pu·MA System.



Clean the membrane, top gaskets and baseplate gaskets with 70% ethanol or IPA .

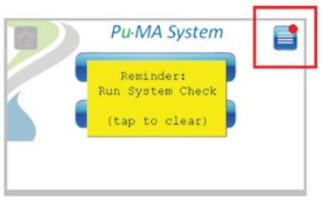


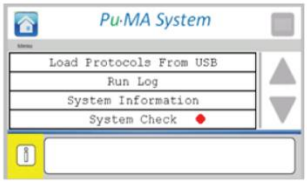
Run “Vacuum Manifold Cleaning Protocol” after every assay.



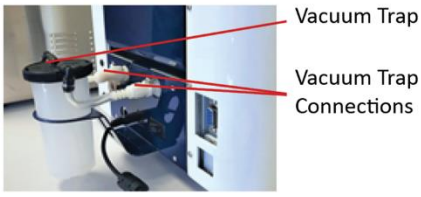
Monthly

Run “System Check Protocol” monthly or when reminded.





Empty and clean the Vacuum Trap



Access short videos and best practices for using the Pu·MA System and 3D Flowchips on our **Support Portal**: **https://proteinfluidics.com/customer-support/**