NanoFabTx™ Microfluidic - Nano, device kit

Protocol for Catalog No. 911593

Introduction

NanoFabTx™ is a set of formulation reagent and device kits from MilliporeSigma designed to effortlessly and rapidly synthesize hydrophobic drugs encapsulated nano- and microparticles. It is based on a highly flexible platform technology that enables researchers to develop nanoformulation of their proprietary hydrophobic drugs without the need for lengthy trial-and-error optimization or expensive instruments. The reagent kits include key reagents and detailed protocols with step-by-step instructions for synthesizing nano- and micro-particle-based formulations. The protocols are optimized for the synthesis of nanoparticles using conventional nanoprecipitation method, which requires minimal resources and laboratory setup, and the most advanced microfluidic method which enables particles with narrow size dispersity and high batch-to-batch consistency. The microfluidic device kits include microfluidic chip, accessories and comprehensive protocols for microfluidic beginners to conduct nano- and microparticle synthesis.

NanoFabTx™ Microfluidic - nano device kit includes pre-assembled microfluidic chip, tubing and required accessories. It is ready for connection with the pump system to conduct nanoparticle synthesis. It is highly recommended to read this document thoroughly before starting the procedure, to ensure the familiarity with the steps. This protocol is intended for microfluidics beginners, and it includes detailed step-by-step instructions, and a troubleshooting section to cover any issues that may arise.

This kit was developed and tested in partnership with Dolomite® Microfluidics. For compatible pumps and microfluidic systems, please visit: https://www.dolomite-microfluidics.com/products/nanofabtx-hardware-solutions/

Disclaimer

NanoFabTx™ Microfluidic - nano device kit is for research use only; not suitable for human use. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Specifications

Storage	Store NanoFabTx™ Microfluidic - nano device kits in a clean dry place at room temperature.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials supplied

Each NanoFabTx™ Microfluidic - nano kit is preassembled and contain these components: *(Identification key indicates how these items are referred in this protocol)*

Catalog Number	Description	Labels in schematics	Quantity
911917	Microfluidic Chip (Micromixing Chip)	chip	1
917494	Tubing: Pre-cut lengths of tubing (250 µm FEP) (preassembled)		
	Junction: T-Junction	T1, T2	2
	Connector: Linear connector 4 way	Input/output connector	2
917230	Interface: Chip Interface H	H Interface	1
	In-line Valve: In-line Valves	V1, V2, V3	3
	Plug: Plug 1.6 mm	Blanking plug	4
916609	End Fittings and Ferrules: End Fittings and Ferrules for 1.6 mm Tubing	L1, L2, and L3	

	Luer lock: Female to female Luer lock (for syringe pumps)		3
917877	Filters: In-line filter		3
	Extra tubing roll (250 µm FEP-10m)		1

Materials required, but not supplied

Catalog Number	Description
909637	NanoFabTx™ PLGA-Nano (required kit for microfluidics protocol)
	Pressurized pumps - Mitos P-Pumps (Cat. No. (https://www.dolomite-microfluidics.com/product/mitos-p-pump/) or syringe pumps (Harvard Apparatus-PHD Ultra) or other syringe pump with precise control over flow rates
	Flow sensors
SLFH025	Syringe filters 0.45µm (for filtering non aqueous solvents like, acetonitrile and DMSO)

Before you start: Important tips for optimal results

Microfluidics method – Reduce blockages with proper cleaning. Clean the microfluidics system after synthesis of each batch of drug-encapsulated nanoparticles. Improper cleaning can result in blockages in the micromixing microfluidics chip and tubing. A well cared-for chip can be used many times; thorough cleaning and proper storage are essential.

Microfluidics method – Prime the tubing and chip. Prime the tubing and the micromixing microfluidics chip before you start nanoparticles synthesis. Priming purges gases from the fluid pathways, conditions the chip surface with the stabilizers, and serves as a check of chemical compatibility for all wetted parts of the system. In addition, priming will help to prevent precipitation of polymers inside the system in the case of backflow, jetting, or chaotic mixing. Precipitation of polymers can irreversibly block the chip.

Procedure

The continuous flow of **microfluidics-based methods** is an advanced method which results in narrow size distribution, enhanced control over each stage of particle fabrication, greater particle yields, ease of scalability, and excellent reproducibility. Figure 1 shows the setup of the preassembled kit components. The schematic also shows how the inlet tubes will be connected to the pumps containing different solutions.

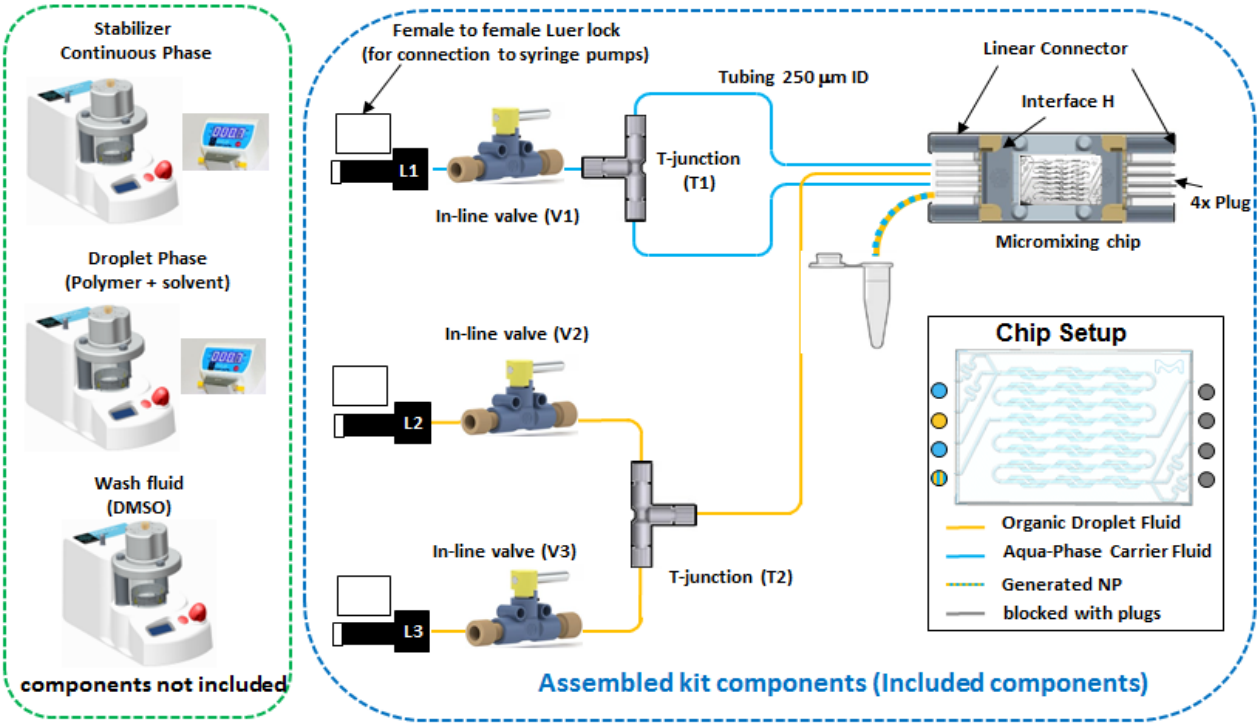

Figure 1: Schematic representation of device kit assembled with the pumps and chip

A. *Assembled Manifold*

- The kit components are preassembled for ease of use.
- To avoid any potential damage to the microfluidics chip while shipping, the chip is housed separately in a gel-pack case inside the kit box and requires assembling as described in section C.
- The recommended pumps are Mitos P-Pumps from Dolomite Microfluidics (Cat. No. https://www.dolomite-microfluidics.com/product/mitos-p-pump/), however the setup can be used with a syringe pump using the luer lock adaptors provided with the kit.
- As shown in the setup illustrated in Figure 1, pump 1 is connected to the channels 1 and 3 (from top) of the microfluidics chip via a T-connector (T1).
- Pump 2 and 3 are connected to the central channel (2nd channel from top) of the microfluidics chip via a T-junction connector (T2).
- In-line valves V1, V2 and V3 are connected to control the flow of fluids from Pump 1, 2 and 3 respectively.
- A 15 cm tube is connected to the output channel (4th channel) for collecting the synthesized nanoparticles.
- The additional luer lock adaptors are provided in the kit in case you want to connect a syringe pump instead of recommended Mitos P-pumps.

B. *Priming the System*

Note: *It is highly recommended to use a priming solvent like DMSO (or any other compatible solvent without any polymers or surfactant system) before attempting to make particles. Priming with a solvent establish a stable droplet formation and avoid precipitation of polymer inside the system in the case of backflow, jetting or chaotic droplet formation. The priming procedure also purges gases from the fluid*

pathways, conditions the chip surface with the choice of solvents or surfactant systems.

- Place a vial (Cat. No. V7130) containing DMSO (10 ml, filtered using a 0.45 µm syringe filter- Cat. No. SLFH025) inside each pump 1, 2 and 3.
- From the preassembled kit, remove the input connector from H-interface by loosening the two screws with hands (figure 2- left panel).
- Using the Mitos Flow Control center (FCC) software (or any other software controlling the pumps), set a pressure of 1000 mbar on Pump 1.
- Open valve V1 to allow the flow of DMSO and within a minute droplet of DMSO will form on the gasket at output positions 1 and 3 (figure 2-middle panel).
- Use a lint free paper to wipe the droplet and observe the formation of fresh droplet at an even rate. (See troubleshooting section in case of uneven droplet formation).
- Close valve V1 and turn off pump 1 using software.
- Repeat the same process with Pump 2 and 3 and observe formation of droplet at position 3.

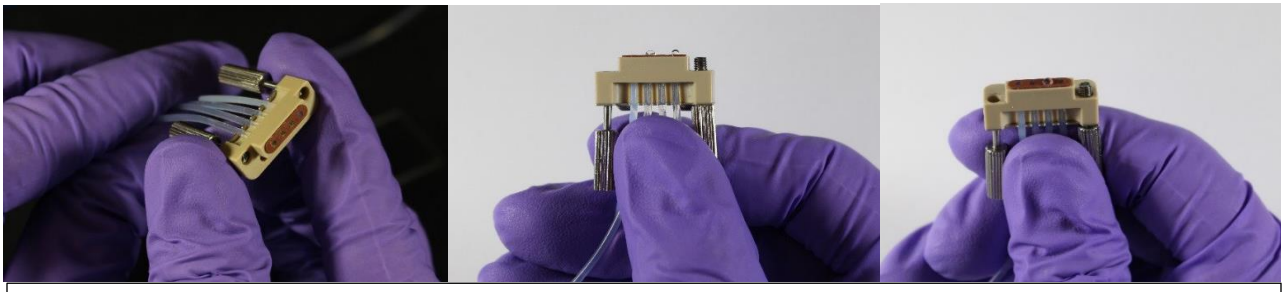

Figure 2: Linear connector (left), droplets of priming fluid forming at the gasket for channel 2 and 4 (middle) and droplet formation for channel 3 (right)

C. *Assembling Microfluidics chip (Final assembly)*

- Remove the chip from the case and place into the H-interface.
- Make sure the chip channels are aligned with the tubing on the input connector.
- Connect input and output connectors to the chip H-interface assembly (figure 3- middle panel).
- Initially, loosely tighten the two screws on each input and output connectors and tighten evenly until finger tight.
  Note: *Ensure the chip is aligned with the connectors as shown in schematics (figure 1). Tighten the interface evenly and securely, while pressing the chip firmly against the H interface. If chip sits in the interface correctly, the gasket will be seen pressing against the chip, with the 4 tubing positions easily identifiable (figure 3-right panel). A poorly connected chip will not show the 4 tubing positions or may show only those on the over-tightened side of the chip. A poor seal between the chip and gasket will likely cause a leak in the system.*

- Place the assembled chip interface on a flat surface or on the stage of a light microscope to observe the flow in the channels.

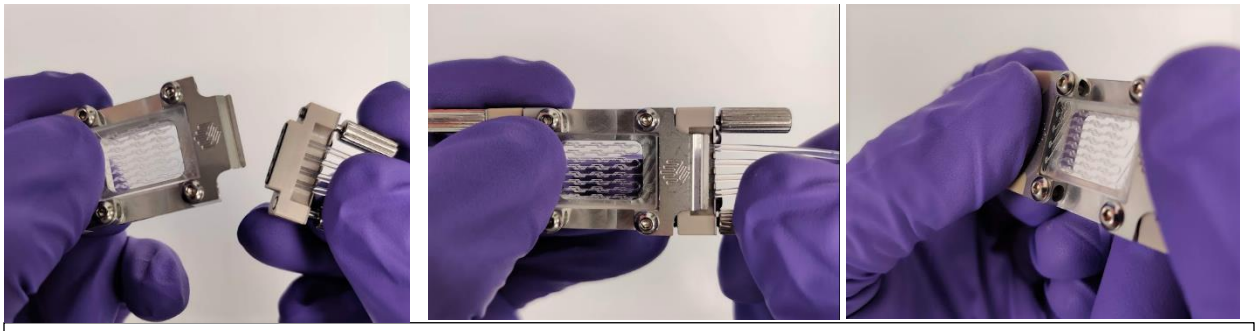

Figure 3: Input line connector and H-interface with chip (left), hand tighten the two screws on input and output connector (middle) and correct alignment between chip and linear connector gasket (right)

D. Clean the microfluidics system

- Cleaning procedure must be followed to remove any remaining precipitates of polymers or any deposited stabilizer.
- Use DMSO to clean the tubing and micromixing microfluidics chip. DMSO is the preferred cleaning solvent, because both the stabilizer and many polymers have high solubility in DMSO.
- Other solvent system which are compatible with the tubing and can dissolve polymers can be utilized for cleaning the system as well.
- Filter 10 ml DMSO through a 0.45 μm syringe filter into each of three vials (Cat. No.V7130).
- Close Valves V1, V2 and V3, place a waste collection vial at the output tubing.
- Place the vials of filtered DMSO in pumps 1, 2 and 3.
- Set the flow rate of pump 1 to 100 μl/min and immediately open Valve 1.
- Follow the previous step for pump 2 and 3 to allow the flow of DMSO.
- Gradually increase the flow rate on all three pumps to 300 μl/min. Run the system for 3 minutes to completely remove any polymer or stabilizer precipitated inside tubing or on a micromixing microfluidics chip.
- When the cleaning process is complete, close valves and use the system's program to immediately stop the flow of the liquids through pumps.
- Remove the vials that contained DMSO vials.
- Disconnect the input/output connectors and remove the micromixing microfluidics chip from the H interface.
- Ensure that the micromixing microfluidics chip is returned to its box for storage, or is placed in another clean, dust-free environment.

Troubleshooting

Due to the numerous connections between microfluidics components, and the narrow flow paths for the fluids, you might encounter issues such as leaks or blockages. This section presents information on issues commonly encountered and their possible solutions

1. Uneven flow in the microfluidics-based method

Possible reason – Uneven flow can be caused by bubbles of air in the system.

Solution –Fluid flowing through the system will clear bubbles within 1–2 min. You can usually see the bubbles passing through the micromixing microfluidics chip. If this approach does not remove the bubbles, sonicate the fluids for 30 min and vent the pressure chamber.

Possible reason – If the flow becomes unstable when the microfluidics system has been in operation for a while, one of the reagent supplies may have run dry or the pick-up tubing might not reach to the bottom of a vial.

Solution – Check that the vials contain enough reagent and that the 250-µm pick-up tubing is long enough to collect from the bottom of each vial.

Possible reason – If none of the above solutions leads to even flow, the software may need to be rebooted.

Solution – Stop all flow, close and reopen the Flow Control Centre software, and restart flow. If this method does not solve the problem, the system may have a blockage. Check for blockages as detailed in the next section.

Possible reason – If the system has no blockages, the flow sensor may not function correctly.

Solution – Replace the flow sensor.

2. Leak in system

Possible reasons – Changes/Fluctuations in system pressure, flow rate, or flow can arise from a leak in the system.

Solution – Before troubleshooting a possible blockage, make sure that all connectors are properly fitted and that the system has no apparent leaks.

3. Blockage of tubing or micromixing microfluidics chip

Possible reasons – During the synthesis of nanoparticles using the microfluidics setup, the introduction of dust fibers, deposition of precipitated polymers/stabilizer, drying of polymers/stabilizers inside the micromixing microfluidics chip or tubing, or improper cleaning procedures can cause blockage in the micromixing microfluidics chip or tubing.
Several indications suggest that a partial or complete blockage has occurred:

a. Consistent flow rate is maintained when a pump is in flow control mode, but the pressure increases.
b. Consistent pressure is maintained when a pump is in pressure control mode, but the flow rate decreases.
c. The instrument software has set changes to the flow rate, but apparent flow rate does not change.
d. The flow is significantly slower than expected.
e. The flow rate fluctuates unexpectedly and affects droplet stability.

Possible reasons – If a partial or transitory blockage is present, the pressure may increase gradually, then suddenly drop as the blockage moves along the flow path, and then increase again when the obstruction becomes lodged.

Solution – Blockages can occur anywhere in the flow path of the system; identifying the location of a blockage is a process of elimination.

Start with the micromixing microfluidics chip, because sometimes blockages (dust or hair) are visible under a microscope. If you find a blockage on the chip, monitor it while you vary the pump pressure to try to dislodge it. If a blockage on the chip cannot be cleared, the chip will need to be replaced.

If you see no physical blockage in the micromixing microfluidics chip, disconnect the chip interface and check whether liquid flows from the tubing. If liquid now flows from the disconnected tubing the blockage is likely either in the chip or the connector was improperly seated against the chip. If the system has a T-connector that splits the flow of a solution into two inputs, check that the flow rates through each input are identical. If the flow is asymmetric, a blockage could be somewhere between the T-connector and the chip. First replace the tubing and see if this fixes the problem; if not, replace the T-connector.

If it is not already apparent which line is blocked, vary the flow rate through continuous and droplet phases one at a time while observing the ends of the tubing. This step will help to identify which line is blocked.

Work your way back through the system, from the chip to the pump, one component at a time, and check for stable flow at each stage. When you find the section that contains the blockage, simply replace it.

The blockage might have occurred because of solid contamination in your solution(s). Although solutions should not require filtration, if they were contaminated after opening, filtering each solution through a 0.45µm syringe filter might prevent the blockage recurring.