



NanoFabTx™ PLGA-Nano, for synthesis of 100 and 200 nm particles

Protocol for Catalog No. 909637

Introduction

NanoFabTx™ nanoformulation reagent kits enable users to encapsulate a wide variety of therapeutic drug molecules for targeted or extended drug delivery without the need for lengthy trial-and-error optimization. **NanoFabTx™** kits provide an easy-to-use toolkit for encapsulating a huge variety of therapeutics in nanoparticles, microparticles, or liposomes. The resulting particles are biocompatible and biodegradable and can be further modified to target specific tissues or to ensure slow and sustained drug release. Drug encapsulated particles synthesized with the **NanoFabTx™** kits are suitable for biomedical research applications such as oncology, immuno-oncology, gene delivery, and vaccine delivery.

The kits minimize laboratory setup with optimized protocols and step-by-step instructions for synthesizing drug-encapsulated nanoparticle-based formulations. Protocols for two different particle synthesis methods are included. One protocol uses the nanoprecipitation method to prepare drug-encapsulated nanoparticles in standard laboratory glassware. A protocol for microfluidics-based synthesis using commercial platforms or syringe pumps is also included. The microfluidics protocol uses **NanoFabTx™** device kits, which provide all the microfluidics chips, fittings and tubing required to get started with microfluidics-based synthesis (compatible microfluidics system or syringe pump required).

NanoFabTx™ PLGA-Nano kit is designed for the synthesis of specifically sized, hydrophobic drug encapsulated poly(lactic-co-glycolic acid) (PLGA) nanoparticles. PLGA is a biocompatible and biodegradable polymer that has been widely used in drug delivery systems for controlled drug release of many different types of therapeutic molecules. The **NanoFabTx™ PLGA-Nano** kit provides reagents and protocols for nanoprecipitation and microfluidics to synthesize 75 nm to 200 nm nanoparticles that enables users to identify the ideal nanoparticle size and drug loading for their research application.

Disclaimer

NanoFabTx™ PLGA-Nano 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™ PLGA-Nano kits at 2-8°C. Protect from light.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials supplied

Each **NanoFabTx™ PLGA-Nano** kit is supplied as follows:

Catalog Number	Quantity
907782	PLGA-Nano (500mg)
907766	Stabilizer-Nano (5g)

Materials required for use with both methods, but not supplied

Catalog Number	Quantity
271004	Acetonitrile
276855	Dimethyl Sulfoxide (DMSO)
Z693472	Magnetic Stirrer
Z266337	Stir bars (40 mm x 8 mm)
V7130	Glass scintillation vials (20 ml capacity)
27024	Glass vials, clear glass (4 ml capacity)
SLFH025	Syringe filters 0.45 μ m (for filtering non-aqueous solutions like polymer/drug solutions, acetonitrile and DMSO)
SLHAR33SS	Syringe filters 0.45 μ m (for filtering aqueous solutions like stabilizer solution)
D9777	Dialysis membrane (12-14kDa cut off)
UFC510024	Amicon Ultra-0.5 Centrifugal Filter Unit – 100kDa
	Deionized water
01885	Docetaxel or hydrophobic drug of choice

Materials required for use with the Dolomite Microfluidics system, but not supplied

Catalog Number	Description
911593	NanoFabTx™ microfluidic – nano device kit
	Pressurized pump system (protocol requires two or three pumps) (e.g. Dolomite Mitos P-Pump). Further information for compatible Dolomite Microfluidic pumps and microfluidic systems can be found at https://www.dolomite-microfluidics.com/products/nanofabtx-hardware-solutions/ .
	Dolomite flow sensors (protocol requires two flow sensors). Further information for compatible Dolomite Microfluidic flow sensors https://www.dolomite-microfluidics.com/products/nanofabtx-hardware-solutions/ .

Materials required for use with syringe pumps, but not supplied

Catalog Number	Description
911593	NanoFabTx™ microfluidic – nano device kit
	Syringe pumps (protocol requires two or three pumps) (e.g. Harvard Apparatus – PHD Ultra pumps)
	Syringes compatible with syringe pumps required (recommended with Hamilton® GASTIGHT® syringes, Cat. No. 26211-U).

Before you start: Important tips for optimal results

Filter solutions. For best results, filter the PLGA solution through a 0.45 μ m syringe filter (Cat. No. [SLFH025](#)) before use. In addition, filter the stabilizer solution through a 0.45- μ m syringe filter (Cat. No. [SLHAR33SS](#)) before use.

Dissolve drug completely. It is essential that the solvent used completely dissolves the drug of interest. Hydrophobic drugs can be especially difficult to dissolve and require careful attention. Hydrophobic solvents such as acetonitrile, DMSO, or DMF are appropriate, but DMSO is recommended because it can dissolve a high concentration of the drug and is compatible with the solvents used to dissolve the PLGA polymer [e.g., acetonitrile, dichloroform (DCM), or acetone]. We recommend you create a concentrated stock solution of your drug using compatible solvents to facilitate dilutions.

Drug loading in nanoparticles. The starting drug concentration must be optimized in order to achieve your specific release rate based on drug selection and application. In this protocol, a starting drug concentration of 10% was used for the model drug docetaxel.

Microfluidics method – Volume of collected nanoparticles. The volume of nanoparticle suspension can be controlled by adjusting the running/collection time. If only a small volume (2-3 ml suspension) of drug-encapsulated nanoparticles is required, run the microfluidics system for 2 to 3 mins. Similarly, to prepare large batches (10 ml), increase the volume of stabilizer solution and drug/polymer solution inside pumps 1 and 2, respectively and collect nanoparticle suspension for 8 to 10 mins. Refer to your pump manual for the recommended maximum vial size.

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-maintained microfluidics chip can be used multiple times through cleaning and proper storage.

Microfluidics method – Prime the tubing and chip. Prime the tubing and the micromixing microfluidics chip before starting nanoparticle 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 reduces or prevents precipitation of PLGA inside the system in the case of backflow, jetting, or chaotic mixing. Precipitation of PLGA can irreversibly block the microfluidics chip.

Microfluidics Method – Using syringe pumps: The flow rates listed in the protocol are optimized using pressurized pumps with flow sensors attached. If a syringe pump is used instead of pressurized pumps, the size of the nanoparticles and percentage of drug loading will deviate from the protocol. To successfully use a syringe pump, follow recommend flow rates listed in **Appendix A**.

When using syringe pumps, gradually increase flow rate in a stepwise fashion to the desired flow rate. Ensure valves are open in the flow path, since back pressure can build up and cause leaks.

Procedure

Two procedures to synthesize PLGA nanoparticles are provided below: **Procedure 1 –nanoprecipitation method** and **Procedure 2 – microfluidics-based method**. **Procedure 1** is a nanoprecipitation method (also known as the solvent displacement or interfacial deposition method) that is widely used to prepare various polymeric nanoparticles because of its scalability, simplicity, and lack of requirements for expensive laboratory instruments. **Procedure 2** is a microfluidics or continuous flow-based technique that results in narrow size distribution, enhanced control over each stage of particle fabrication, greater particle yields, ease of scalability, and excellent reproducibility. This microfluidics-based protocol uses the rapid micromixing method to synthesize drug-encapsulated nanoparticles.

This procedure for hydrophobic drug encapsulation was developed and optimized to achieve a desired size range using docetaxel (DTX) as a model. The *NanoFabTx™* PLGA-Nano protocol can be modified for the hydrophobicity and size of your hydrophobic drug of interest to achieve a desired size and encapsulation efficiency. The suggestions below are suggested as a guide for your own optimization.

Prepare reagents

Reagent preparation instructions are for use with both the nanoprecipitation method (Procedure 1) and the microfluidics method (Procedure 2).

1. Prepare 1 ml of 5% (w/v) PLGA stock solution

- Weigh 50 mg of PLGA-Nano from the kit in a glass vial (Cat. No.[27024](#)).
- Add 1 ml of acetonitrile to the vial using a pipette and cap securely.
- Vortex solution gently for 1-2 mins to completely dissolve the polymer. The final solution should be a clear transparent solution. DO NOT vortex or shake vigorously.
- Filter the PLGA stock solution through a 0.45- μ m syringe filter (Cat. No. [SLFH025](#)) before use.

2. Prepare 50 ml of 1% (w/v) stabilizer solution

- Place a clean stir bar (Cat. No. [Z266337](#)) into a glass 100 ml glass beaker.
- Add 50 ml deionized water.
- Weigh 500 mg of Stabilizer-Nano from the kit and transfer to the glass beaker.
- Place the beaker on a heated magnetic stir plate and stir the solution gently. While stirring, warm the solution to 50–60 °C to completely dissolve the stabilizer. The final solution should be a clear transparent solution. You may also use a heat gun to warm the solution.
- Let the stabilizer solution cool down to room temperature and adjust the total volume of solution to 50 ml by adding required volume of DI water if necessary.
- Filter the stabilizer stock solution through a 0.45 μ m syringe filter before use. The stabilizer stock solution can be stored at room temperature for up to 48 hrs.

3. Prepare 1 ml of 25 mg/ml drug stock solution

Note: Docetaxel (DTX) (Cat. No. [01885](#)) was used as a model hydrophobic drug in this protocol. Using a different drug will require further optimization.

- For DTX, weigh out 25 mg and transfer to a glass vial (Cat. No. [27024](#)). The amount needed of your drug will depend on drug characteristics, your desired release rate and application.
- For 25 mg of DTX, add 100 μ l of DMSO using a pipette. For other drugs, add the minimal volume of DMSO needed for dissolving. Cap securely. Dissolve the drug of interest by gently swirling the vial by hand.
- For DTX, add 900 μ l of acetonitrile to bring the total volume to 1 ml. For other drugs, add enough acetonitrile to bring the total volume to the amount you would like to encapsulate in nanoparticles.

Procedure 1 – Nanoprecipitation method to synthesize PLGA nanoparticles

A Prepare reagents

See Prepare reagents instructions above to make stock solutions.

1. Prepare drug/polymer solution (see **Table 1**)

- After selecting desired nanoparticle size range from **Table 1**, prepare 1 mL drug/polymer solution in a glass vial (Cat. No. [27024](#)) by mixing together the indicated volumes of PLGA polymer stock solution, drug stock solution, and solvent.
- Cap the vial and swirl gently by hand to completely mix the solutions.



Table 1: Preparation of drug/polymer solution for nanoprecipitation of desired nanoparticle size

Selected nanoparticle size	Volume PLGA polymer stock solution (5% w/v)	Volume drug stock solution (25 mg/ml)*	Volume of solvent (acetonitrile)	Final volume of drug/polymer solution	Total polymer concentration (% wt/v)	Total drug concentration (% drug / wt of polymer)
75-100 nm	150 µl	30 µl	820 µl	1 ml	0.75	10
150-200 nm	600 µl	120 µl	280 µl	1 ml	3	10

***Note:** Table recommendations based on model drug, DTX. For other drug types please see "drug loading in nanoparticles" note in previous section.

B Synthesize drug-encapsulated nanoparticles

1. **Encapsulate the drug in nanoparticles**
 - Place a clear stir bar (Cat. No. [Z266337](#)) into a glass vial (Cat. No. [V7130](#)).
 - Add 10 ml of the 1 % (w/v) stabilizer stock solution.
 - Place the uncapped vial on a magnetic stir plate and stir solution at 1000 rpm at room temperature. Continue to stir the solution during the next step.
 - Use a pipette or a syringe to inject 1 ml of the drug/polymer solution into the stabilizer solution. Continue to stir the solution during the addition.
 - The stabilizer solution will become slightly translucent as you add the drug/polymer solution. The change in appearance indicates rapid nucleation and formation of drug-encapsulated nanoparticles.

Note: A slight translucency is a good indicator of nanoparticle formation, however it is important to carefully observe the reaction for any visible precipitates floating in solution. Visible precipitates can be indicative of formation of large polymer precipitates, or drug precipitates.

 - Continue to stir the solution for 30 min to complete synthesis of the drug-encapsulated nanoparticles.
2. **Remove excess stabilizer, solvent, and non-encapsulated drug**
 - Transfer the drug-encapsulated nanoparticle suspension to a 12-kDa cut-off cellulose membrane (Cat. No. [D9777](#)).
 - Dialyze against 4L deionized water for 4-6 h at room temperature, change water after 30 minutes and 1 hour.
 - Transfer the dialyzed drug-encapsulated nanoparticles solution to a glass vial and store at 2-4 °C until needed.
3. **Measure size of drug-encapsulated nanoparticles**
 - Measure the size of the drug-encapsulated nanoparticles with a dynamic light scattering instrument and transmission electron microscopy (TEM).

Procedure 2 – Microfluidics-based method to synthesize PLGA nanoparticles

A. Set up the microfluidics system

This microfluidics protocol is designed for use with the corresponding *NanoFabTx™ Microfluidic - Nano* Device kit (Cat. No. [911593](#)) which contains all required device components for synthesizing nanoparticles and a detailed protocol for use with a Dolomite Microfluidics system or syringe pumps. Please follow the protocol with *NanoFabTx™ Microfluidic - Nano* Device kit (Cat. No. [911593](#)) to set up the microfluidics system. **Figure 1** shows a schematic of the microfluidics setup and tubing connections for a Dolomite Microfluidics system and **Figure 2** shows a schematic for connection to syringe pumps.

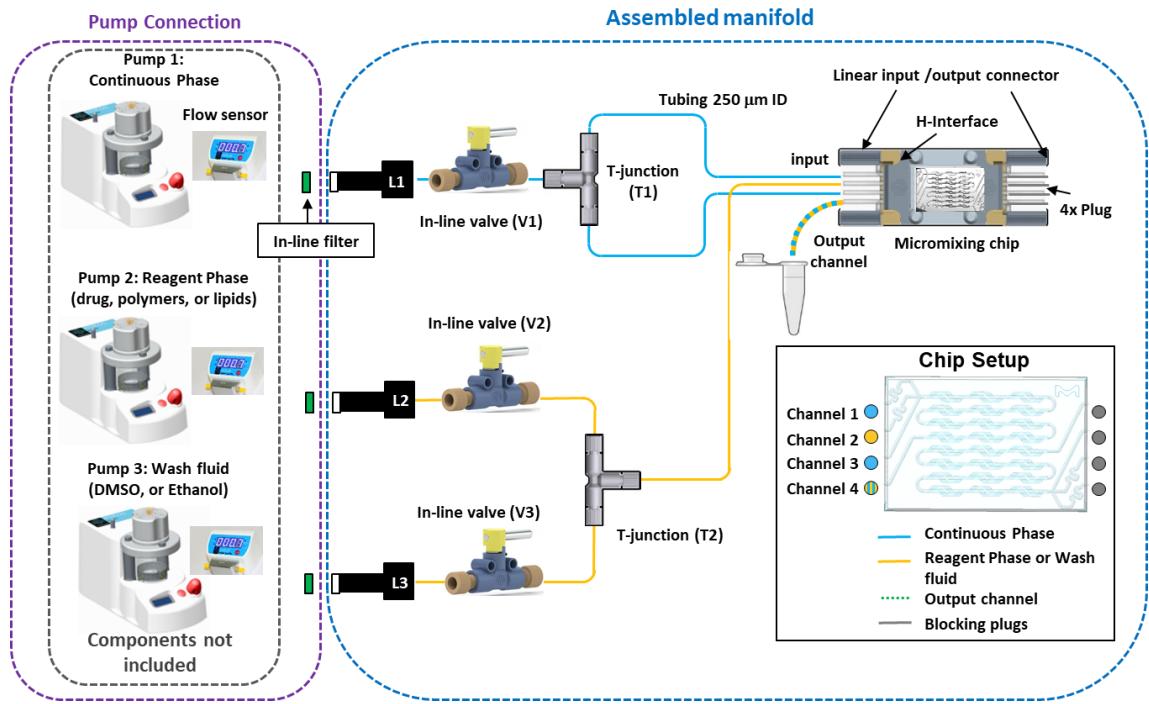


Figure 1: Schematic of the microfluidics setup for the Dolomite Microfluidics system. The manifold is supplied preassembled. The microfluidics chip is packaged separately. In-line filters are supplied for connection to microfluidic pumps. More information can be found in the corresponding NanoFabTx™ Microfluidic - Nano Device kit (Cat. No. [911593](#)).

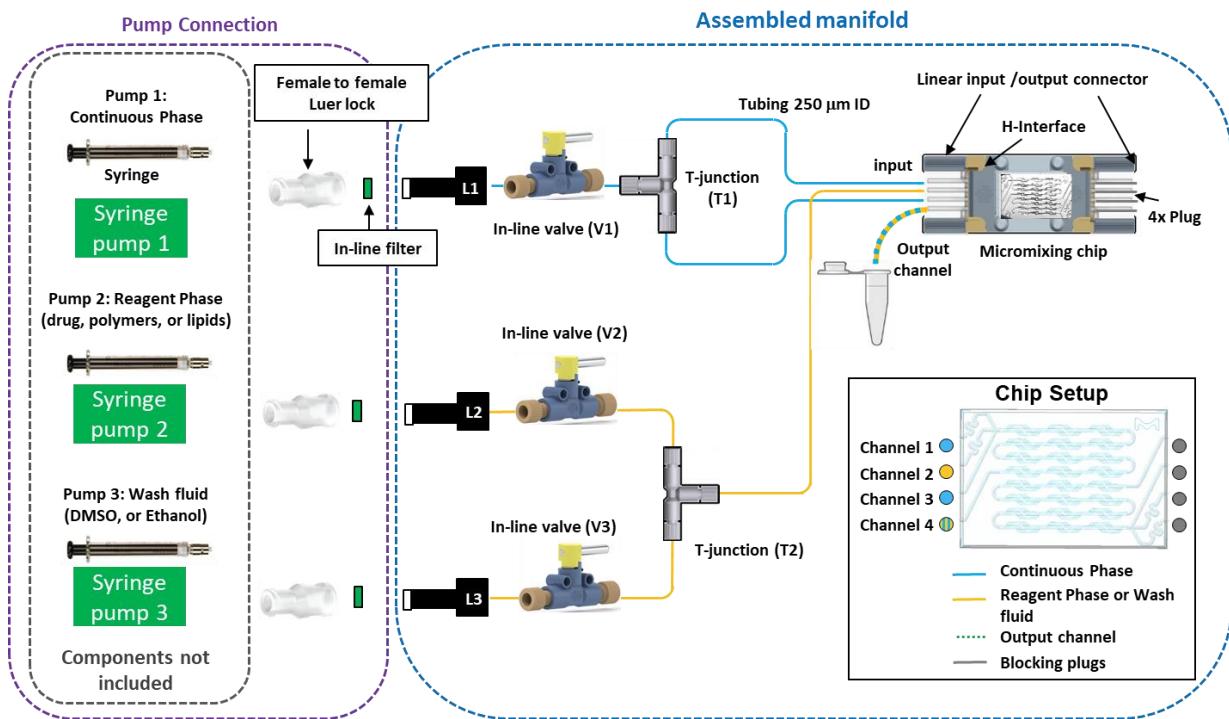


Figure 2: Schematic of the microfluidics setup for a syringe pump. The manifold is supplied preassembled. The microfluidics chip is packaged separately. In-line filters and luer locks are supplied for connection to syringe pumps. More information can be found in the corresponding NanoFabTx™ Microfluidic - Nano Device kit (Cat. No. 911593).

B. Prepare reagents

1. Prepare drug/polymer solution (see Table 2)
 - After selecting desired nanoparticle size range from **Table 2**, prepare 10 mL drug/polymer solution in a glass vial (Cat. No. [V7130](#)) by mixing together the indicated volumes of PLGA polymer stock solution, drug stock solution, and solvent.
 - Cap the vial and swirl gently by hand to completely mix the solutions.

Table 2: Preparation of drug/polymer solution for microfluidic synthesis of desired nanoparticle size

Selected nanoparticle size	Volume PLGA polymer stock solution (5% w/v)	Volume drug stock solution (25 mg/ml)*	Volume of solvent (acetonitrile)	Final volume of drug/polymer solution	Total polymer concentration (% wt/v)	Total drug concentration (% drug / wt polymer)
75-100 nm	500 µl	100 µl	9.4 ml	10 ml	0.25	10
150-200 nm	2 ml	400 µl	7.6 ml	10 ml	1	10

*Note: Table recommendations based on model drug, DTX. For other drug types please see "drug loading in nanoparticles" note in previous section

2. Prepare stabilizer solution (see Table 3)

- After selecting desired nanoparticle size range from **Table 3**, prepare 10 mL stabilizer solution in a glass vial (Cat. No. [V7130](#)) by mixing together the indicated volumes of stabilizer stock solution and DI water.
- Cap the vial and swirl gently by hand to completely mix the solutions.

Table 3: Preparation of stabilizer solution for microfluidic synthesis of desired nanoparticle size

Selected nanoparticle size	Volume stabilizer stock solution (1% w/v)	Volume of solvent (DI water)	Final volume of stabilizer solution	Final concentration of stabilizer (%w/v)
75-100 nm	5 ml	5 ml	10 ml	0.5
150-200 nm	1 ml	9 ml	10 ml	0.1

*Note: Table recommendations for stabilizer solution is based on model drug, DTX.

C. Synthesize drug-encapsulated nanoparticles

1. Assemble microfluidics system

- Assemble the microfluidics system as described in protocol with *NanoFabTx™ Microfluidic - Nano Device* kit (Cat. No. [911593](#))

Note: The synthesis of nanoparticles using the Dolomite Microfluidics system or syringe pump can be carried out in either a three-pumps (as shown in **Figure 1** and **Figure 2**) or two-pumps configuration (pump 1 and 2 only). In the three-pumps configuration, a vial of priming solution is kept in pump 3 throughout the process. Pump 3 can be used for the washing step without the need for swapping the vials in pump 2 when using a two-pumps configuration. For washing, simply close valve V1 and V2 and open valve V3 and start the flow of DMSO using the pump software.

2. Insert priming solvent DMSO into microfluidics system

- As shown in the setup illustrated in **Figure 1** and **Figure 2**, place a vial (Cat. No. [V7130](#)) of DMSO inside pump 1. Pump 1 is connected to channels 1 and 3 of the micromixing microfluidics chip.
- Place another vial (Cat. No. [V7130](#)) of DMSO inside pump 2 or pump 3. Pump 2 and pump 3 are connected to the channel 2 of the micromixing microfluidics chip via T-junction (T2).
- Keep valves V1, V2, and V3 closed. They connect to pump 1, pump 2, and pump 3 respectively.

3. Prime the system

- Place a waste collection vial at the output channel to collect waste generated during setup and priming.



- Open valve V2 to flush the assembled manifold with the DMSO (no PLGA or drug) by setting a flow rate of 100 $\mu\text{l}/\text{min}$ for pump 2 using the Flow Control Center software for the Dolomite microfluidics system or using the syringe pump interface. Keep valve V3 closed if using the two-pumps configuration.

Note: When using syringe pumps, gradually increase flow rate in a stepwise fashion to the desired flow rate. Ensure valves are open in the flow path, since back pressure can build up and cause leaks.

- Close valve V2 and open valve V1 to flush the assembled manifold with DMSO by setting a flow rate of 100 $\mu\text{l}/\text{min}$ for pump 1. For further details on priming the system refer to the device kit protocol (Cat. No. [911593](#))

4. Encapsulate drug in nanoparticles

- Place a vial (Cat. No. [V7130](#)) containing 10 ml stabilizer solution inside pump 1 (refer to **Table 3 for preparation**) and the vial (Cat. No. [V7130](#)) of drug/polymer solution inside pump 2 (refer to **Table 2 for preparation**).
- Check that both valves V1, V2, and V3 are closed. Always keep valve V3 closed when using the two-pump configuration.
- Open valve V1 and set the flow rate for the stabilizer solution to 500 $\mu\text{l}/\text{min}$ using the Flow Control Center software of the Dolomite microfluidics system or set the flow rate according to **Appendix A** on the syringe pump interface.
- The flow rate of the stabilizer solution will stabilize within a few seconds.
- Set the flow rate for pump 2 to 200 $\mu\text{l}/\text{min}$ of the drug/polymer solution if using the Dolomite microfluidic system or set the flow rate according to **Appendix A** on syringe pump interface, and open valve V2.
- The flow rates of both solutions will stabilize within a few seconds. Optional: fluid flow of the two solutions can be visualized by a high-speed microscope.
- After the flow rates of the two solutions have stabilized, replace the waste collection vial with a sample collection vial at the output channel and collect the drug-encapsulated nanoparticle suspension. The flow rates in this protocol will yield 8–10 ml of the drug-encapsulated nanoparticle suspension in approximately 10 minutes.
- When you have collected the desired volume of the drug-encapsulated nanoparticle suspension, transfer the output channel tubing to the waste collection vial, close valves V1 and V2, use the Flow Control Center software or syringe pump interface to stop fluid flow, and remove the solution vials from pump 1 and pump 2.
- Remove excess stabilizer, solvent, and non-encapsulated drug from your nanoparticle suspension as described in previous nanoprecipitation method section.
- Measure size of drug-encapsulated nanoparticles with a dynamic light scattering instrument and TEM.
- Clean the microfluidics system after each use using the method below. Improper cleaning can result in chip and tubing blockages.

D. **Clean the microfluidics system**

- Follow this cleaning procedure after each run to remove any remaining polymer precipitates or deposited stabilizer.
- Use DMSO to clean the tubing and micromixing microfluidics chip. DMSO is the preferred cleaning solvent, because both the stabilizer and PLGA have high solubility in DMSO.
- Filter 10 ml DMSO through a 0.45 μm syringe filter into each of two vials (Cat. No. [V7130](#)).
- Close valves V1, V2, and V3 and place a waste collection vial at the output channel tubing.
- Place the vials of filtered DMSO in pumps 1 and 2.

- Open valve V1 and set the flow rate of pump 1 to 100 $\mu\text{l}/\text{min}$.
- Set the flow rate of pump 2 to 100 $\mu\text{l}/\text{min}$ and immediately open valve V2.

Note: If using a three-pump configuration, washing is not required for pump 3.

- Gradually increase the flow rate on both pumps to 300 $\mu\text{l}/\text{min}$. Run the system for 3 minutes to completely remove any PLGA or stabilizer precipitated inside tubing or on micromixing microfluidics chip.
- When the cleaning process is complete, close valves V1 and V2 and use the software or pump interface to immediately stop the flow of the liquids through pumps 1 and 2.
- Remove the DMSO vials.
- Disconnect the linear 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.

Quantify drug loading

The method for encapsulated drug quantification varies depending on the drug. This is a generalized HPLC method (for guidance only) that has been optimized for the model drug docetaxel (DTX). Modification of the below methods is required based on the encapsulated drug and method chosen for quantification.

A. Extract non-encapsulated drug from as-prepared nanoparticles

- The as-prepared nanoparticle suspension contains both free (non-encapsulated) drug and drug-encapsulated inside nanoparticles. An Amicon Ultra-0.5 centrifugal Filter Unit (100kDa) (Cat. No. [UFC510024](#)) is recommended to separate the non-encapsulated drug from the nanoparticles
- Add 500 μl of the nanoparticle suspension to the Amicon Ultra-0.5 centrifugal Filter Unit.
- Centrifuge the filter unit containing the nanoparticle suspension at 11000 rpm for 45 minutes to completely remove the solution.
- Nanoparticles will remain in the filter tube and the filtrate containing the non-encapsulated drug is collected in the collection tube.
- Take 200 μl of filtrate from collection tube, containing free drug and add equal volume acetonitrile. (Solvent selection is for HPLC quantification, the recommended mobile phase for docetaxel is 50:50 acetonitrile/water). You may need to determine the optimal mobile phase for your drug of interest.
- Quantify the free-drug content in solution using HPLC.

Note: The acetonitrile/water mixture is the mobile phase for docetaxel for HPLC. If you are using another mobile phase, you can dilute the filtrate in your mobile phase for HPLC quantification

B. Quantification analysis

- *Encapsulation efficiency (EE%)* is the percentage of drug that is encapsulated into the nanoparticles.

Encapsulation efficiency is calculated by:

$$EE (\%) = \frac{\text{Total drug added} - \text{free drug}}{\text{total drug added}} \times 100$$

- *Loading capacity (LC%)* is the amount of drug loaded per unit weight of polymer and indicates the percentage of mass of polymer that contains encapsulated drug.

Loading capacity is calculated by:

$$LC(\%) = \frac{\text{Total amount of encapsulated drug}}{\text{Total weight of polymer}} \times 100$$

Troubleshooting

Detailed troubleshooting on the microfluidics setup is provided in the troubleshooting guide included in the *NanoFabTx™ Microfluidic - Nano, device kit* (Cat. No. [911593](#)). Due to the numerous connections between microfluidics components, and the narrow flow paths for the fluids, you may encounter leaks or blockages. This section presents information on and potential solutions for commonly encountered problems.

1. Nanoparticles are not in the defined size range

Possible cause – This protocol is optimized for synthesis of PLGA nanoparticles in the size range as described in **Table 1** and **Table 2** using the model drug, docetaxel. If you encapsulate a different drug in PLGA nanoparticles, the size of your nanoparticles may vary from the size range reported here.

Solution – You can tune the size of the nanoparticles by varying the concentrations of PLGA polymer and drug. In general, increasing the PLGA concentration yields larger nanoparticles. Further, you can tune the size of nanoparticles by changing the flow rate when using the microfluidics-based method.

Possible cause – Procedure 2 is optimized for synthesis of PLGA nanoparticles in the size ranges as described in **Table 1** and **Table 2** using the Dolomite microfluidic system or the recommended syringe pumps. The flow rates listed in the protocol are optimized using pressurized pumps with flow sensors attached. If you use different flow rates, or alternative syringe pumps, the size of your nanoparticles may vary from the size range reported here.

Solution – If syringe pumps are used instead of pressurized pumps from Dolomite, follow **Appendix A** for the correct flow rates using the recommended syringe pumps. If you use another type of syringe pump, you will have to optimize flow rates to achieve the desired nanoparticle size.

2. Polydisperse nanoparticles

PDI is the standard deviation of the particle diameter distribution divided by the mean particle diameter. PDI is used to estimate the average uniformity of a particle solution; higher PDI values correspond to a greater size distribution in the nanoparticle sample. A sample is considered monodisperse when the PDI value is less than 0.1.

Possible cause – Polydisperse samples can result if the stirring rate varies between batches in the nanoprecipitation method.

Solution – Maintain the same stirring rate and use the same stir bars with every batch to minimize polydispersity.

Possible cause – In addition, polydisperse samples can result if the rate of addition of the drug/polymer reagent solution to the stabilizer solution varies during addition or between batches when using the nanoprecipitation method.

Solution – Use a syringe pump to control the rate of addition of the drug/polymer solution when using the nanoprecipitation method.

Possible cause – In the microfluidics method, polydisperse samples can occur if the flow in the tubing or micromixing microfluidics chip is uneven or blocked.

Solution – The next sections provide tips to minimize uneven flow or remove blockages.

3. Uneven flow in the microfluidics-based method

Possible cause – 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 solutions for 30 min and vent the pressure chamber.

Possible cause – If the flow becomes unstable when the microfluidics system has been in operation for a while, one of the solution 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 cause – 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 cause – If the system has no blockages, the flow sensor may not function correctly.

Solution – Replace the flow sensor.

4. Leak in system

Possible cause – Changes/fluctuations in system pressure or flow rate 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.

5. Blockage of tubing or micromixing microfluidics chip

Possible cause – 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 cause – 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 of the solutions 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 may have occurred because of particulate contamination in your solution(s). Refilter solutions through a $0.45\mu\text{m}$ syringe filter before use.

Appendix A: Syringe pump flow recommendations

Microfluidics Method – Using syringe pumps: The flow rates listed in the protocol are optimized using pressurized pumps with flow sensors attached. If a syringe pump is used instead of pressurized pumps, the size of the nanoparticles and percentage of drug loading will deviate from the protocol. To successfully use a syringe pump, follow recommend flow rates listed in **Table 4**.

Table 4: Flow rates required for microfluidic nanoparticle synthesis using commercially available pump systems

Pump type	Brand	Drug/polymer solution flow rate for pump 2 ($\mu\text{l}/\text{min}$)	Stabilizer solution flow rate for pump 1 ($\mu\text{l}/\text{min}$)
Pressurized pumps	Dolomite Mitos P-Pumps	200	500
Syringe pumps	Harvard Apparatus – PHD Ultra pumps	470	500

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