

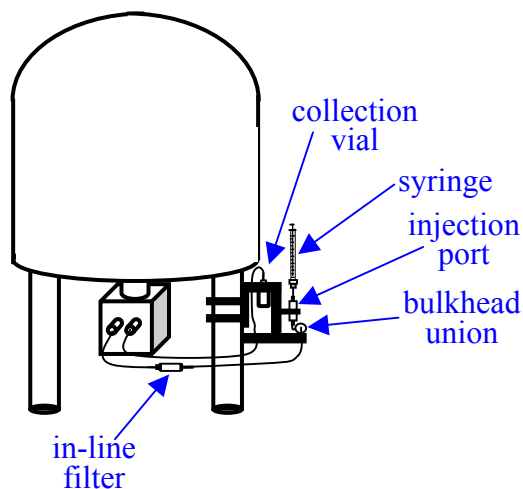
Technical Bulletin G0020: Manual Injection Techniques and Procedures

Rev 12/04

MIM (Manual Injection Module)

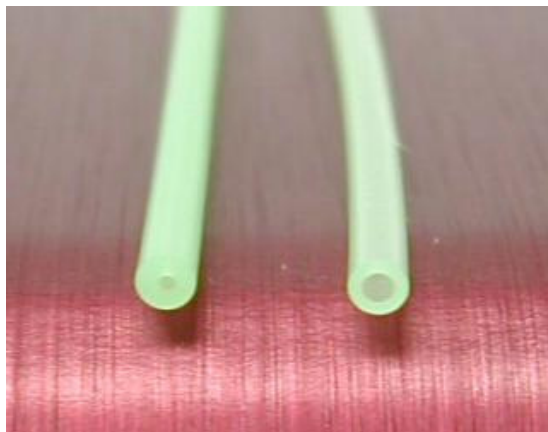
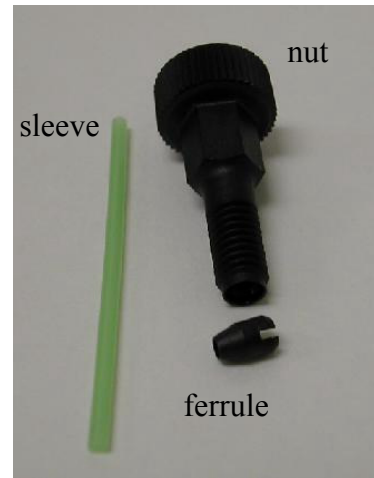
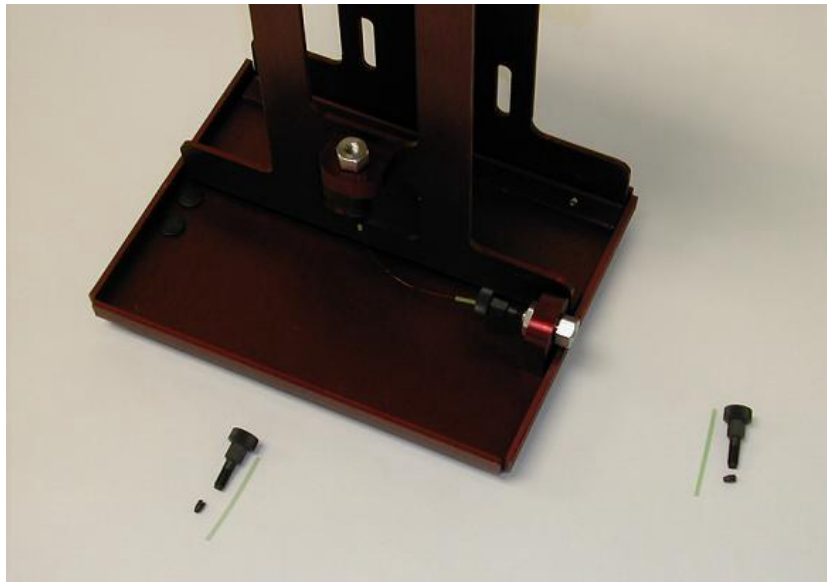
The Manual Injection Module (MIM) is a syringe-based sample management platform used to manually inject microliter-volume samples into the CapNMR™ probe. Shown in the diagram immediately below, the input feed capillary of the probe connects to the MIM through a low dispersion bulkhead union that is plumbed to an injection port. A syringe is inserted into the injection port for sample injection. A collection vial is provided for sample recovery.

The MIM can be positioned to best accommodate the convenience of the user. Typical positions include floor-mounted beneath the magnet and strap-mounted to the magnet leg.



Fluidic Connections

Capillary tips must be free from dirt and cleanly cut to ensure robust fluidic connection. Additional information on capillary cutting techniques can be found in Protasis/MRM Technical Bulletin G0112 – ‘Fluidic Connections’ at www.microNMR.com. The fluidic components required to secure the feed capillary of the probe to the MIM are shown in the figure below. A black, slotted high-pressure ferrule surrounds a green polymer sleeve into which the feed capillary is inserted. A black threaded nut is used to apply sufficient pressure to the ferrule to maintain fluidic seal and mechanical support of the capillary in the bulkhead union. A nut/ferrule/sleeve geometry is also employed to provide fluidic seal and mechanical support of the syringe needle in the injection port. Both sleeves employed in the MIM have 1/16” outer diameter, but differ in inner diameters (see specifications and part numbers below).



Nut and Ferrule

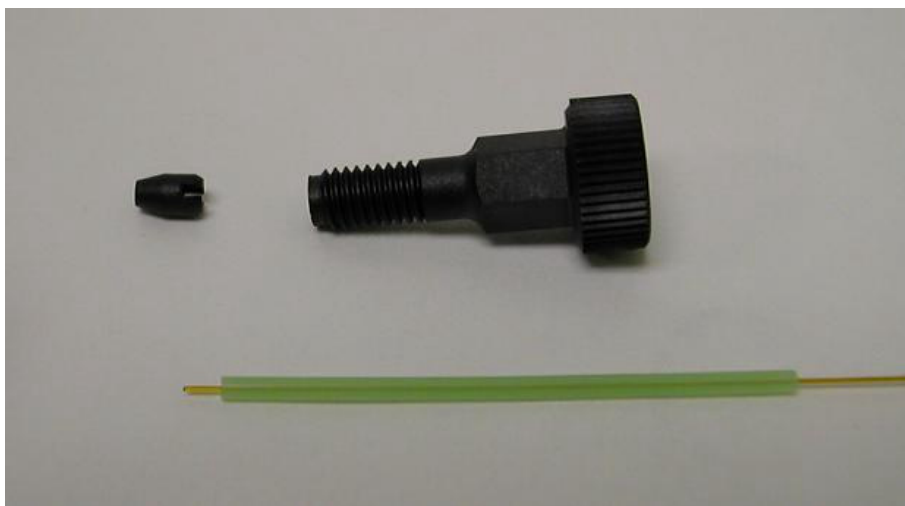
- part # F-287

Large I.D. Sleeve

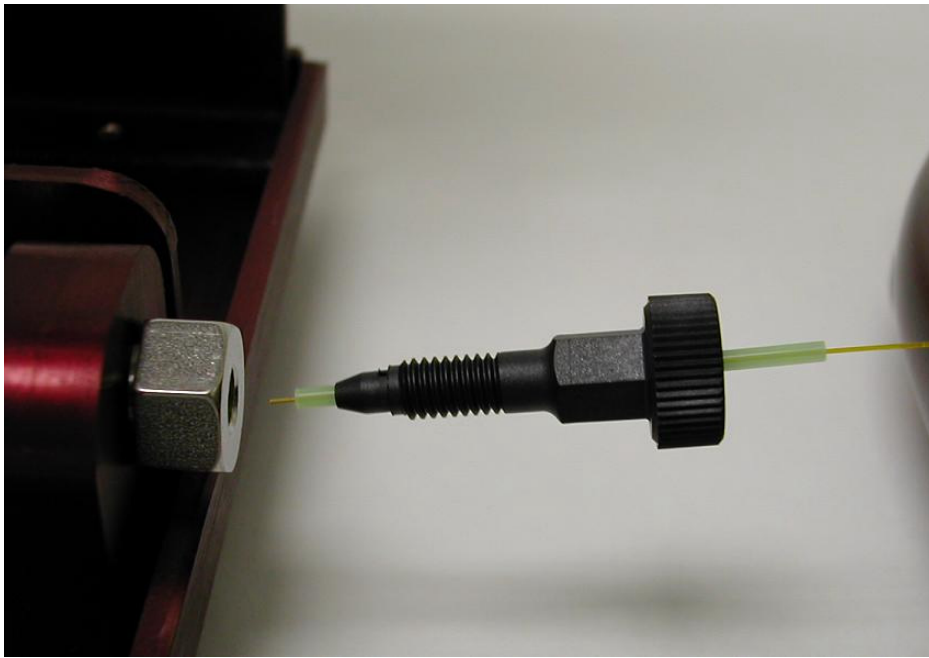
- part # F-247
- connects syringe and Injection port
- accommodates 22 gauge needle

Small I.D. Sleeve

- part # F-242
- connects capillary and bulkhead union
- accommodates 360 μ m O.D. capillary

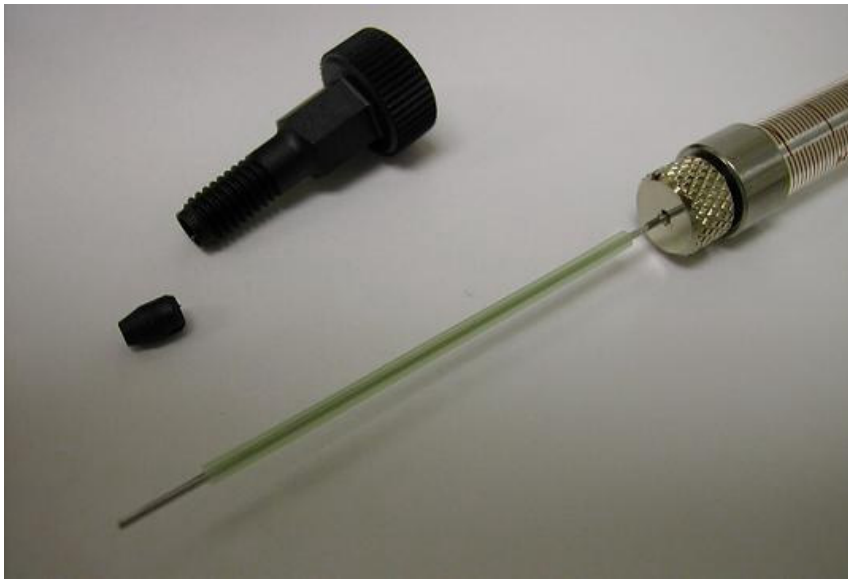


For fluidic connection, slide the polymer sleeve (shown is F-242) onto the end of the capillary. The nut slides over the sleeve, non-threaded end first, followed by the ferrule. The tapered end of the ferrule faces away from the nut. The capillary should protrude slightly from the tip of the green sleeve, and the sleeve should protrude slightly from the ferrule.



The assembly of nut, ferrule, sleeve, and capillary is now inserted into the bulkhead union. The tip of the capillary should securely seat against the internal stop of the bulkhead union. To ensure contact, you may apply light pressure using your fingers to ensure that the capillary and sleeve remain seated against the stop in the union while tightening down the nut.

Sample Injection



The nut, ferrule, and sleeve assembly for the needle of the injection syringe is shown. The (larger i.d., F-247) sleeve fits snugly over the 22 gauge needle. The needle and sleeve assembly fit into the nut in a manner analogous to that explained above for the bulkhead union.



The needle, sleeve, and nut assembly is inserted into the injection port and the nut is subsequently tightened. Note that this is a reusable connection. Several millimeters of the tip of the sleeve should be trimmed away with a razor blade after approximately 4 injections to maintain leak-free operation.

A black holding bracket is provided at the top of the MIM to secure the body of the syringe.

Injection Protocol

It's important to remember that the nut must be removed from the injection port prior to removing or inserting the syringe needle and sleeve. The procedure is illustrated above. Insertion and removal of the syringe needle while the nut and/or sleeve remain in the injection port may result in occurrence of air bubbles and inaccurate sample positioning.

Steps required for accurate sample injection

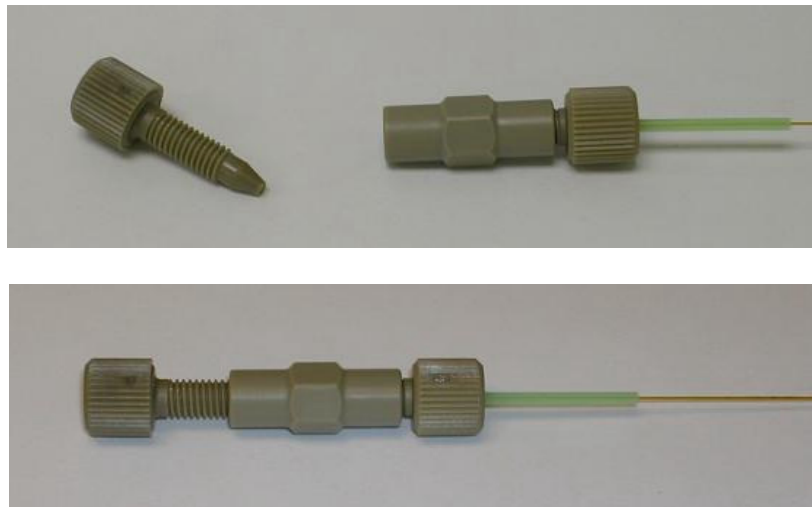
- 1) Rinse fluidic pathway with solvent of choice to ensure that the fluidic pathway is clean. This can be done using the 50uL syringe provided in the installation kit.
- 2) Remove the 50uL syringe, sleeve, and nut from the injection port, as described above.
- 3) Pull up the volume of sample desired for injection into the 10uL syringe.
- 4) Slide the nut/ferrule/sleeve over the syringe tip.
- 5) Place the assembly into the injection port. The syringe needle tip should make contact with the bottom of the injection port.
- 6) Tighten* the nut while ensuring the syringe is seated against the injection port stop. (Rotating the needle inside the port while tightening is a good way to ensure the seat).
- 7) Inject the sample.
- 8) Remove the 10uL syringe/nut/sleeve assembly from the injection port, and re-insert the 50uL syringe with sufficient solvent calibrated to serve as push solvent. Information on push solvent calibration can be obtained on the knowledge base at www.microNMR.com in Technical Bulletin A0210 – 'Manual Calibration of Push Volume.'

* Obtaining fluidic seal using mechanical pressure fit of the plastic sleeve around the metal requires some practice and experience. Generally one should attempt to firmly tighten the nut by hand. If droplets are observed leaking out of the top of the sleeve, try using a 1/4" wrench to tighten the nut, being careful not to remember that plastic threads can strip if over-tightened, and the plastic nut can break.

9) Inject the calibrated amount of push solvent to accurately position the sample in the NMR flowcell.

10) *Leave the 50uL syringe in the injection port during NMR acquisition.* This maintains fluidic seal on the inlet end of the feed capillary during NMR acquisition.

11) If NMR acquisition will consume more than approximately 20 minutes of time, it is strongly recommended that the outlet end of the feed capillary be sealed to prevent evaporation and possible sample movement due to air pressure fluctuations. Shown below, the P-550 plug, P-779-01 union, F-331N nut, and F-242 sleeve are utilized to secure fluidic seal on the outlet leg from the probe. All are included in the Protasis/MRM MIM installation parts kit.



Carryover

One should rinse the probe between injections using the 50uL solvent syringe. A minimum of 5 flowcell volumes (i.e. 25uL for a 5uL CapNMR probe), and more preferably 10 flowcell volumes, is recommended to bring carryover to less than 1%. The solvent in which the sample is dissolved should be employed. Solvent changeover should be accommodated using a path of miscible solvent migration from starting solvent to desired solvent. A chart of miscible solvents can be found in Technical Bulletin KO520 in the knowledge base at www.microNMR.com. In more extreme cases where special procedures were employed to bring the sample into solution (i.e. vortexing, heating, sonicating, etc.) a rinse of 100uL – 150uL is recommended to eliminate carry-over.

Additional Notes

Syringes:

Always use Gastight® syringes. Protasis/MRM Corp recommends using Hamilton brand gastight syringes.