

Aquapel Treatment of Drop-seq Devices

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These instructions are based on the protocol described in: Single-cell analysis and sorting using droplet-based microfluidics, Mazutis *et al.*, Nat Protoc. 2013 May;8(5): 870-91.

Protocol Summary:

Inject microfluidic devices with Aquapel. Let sit for approximately 30-60 seconds. Expel Aquapel with forced air. Flush channels with Fluorinert FC-40 oil to remove residual Aquapel. Expel Fluorinert with forced air. Bake at 65°C for 20 minutes.

Preparation of Aquapel Water-repellent Solution:

The Aquapel solution is supplied in a glass ampule embedded in plastic cartridge with a thick felt pad. Carefully cut the pad and remove the ampule (Figure 1).

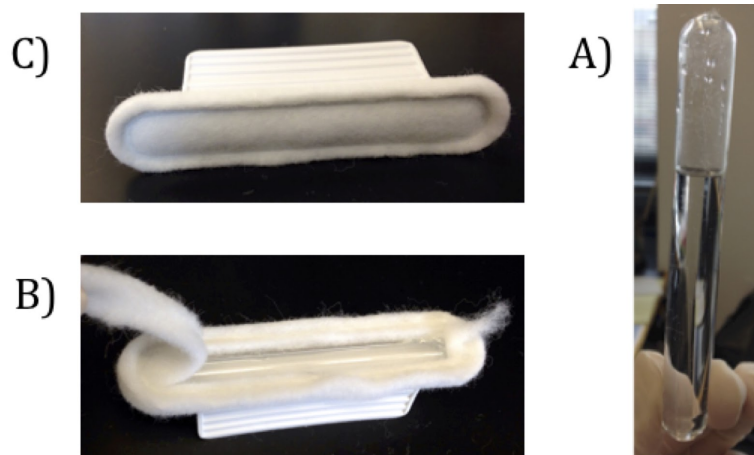


Figure 1: A) Aquapel cartridge. B) Aquapel cartridge with felt pad cut away. C) Aquapel ampule extracted from cartridge housing.

Place the ampule at the bottom of a 50-mL Falcon-type conical plastic tube and carefully break the ampule inside the tube. Transfer the Aquapel solution into a 5-mL glass, gas-tight syringe, while leaving the broken glass in the 50-mL plastic tube. **Critical step:** Expel the air bubbles from the syringe. Upon prolonged contact with air, Aquapel precipitates and loses its functionality. Aquapel can be stored for up to ~2 weeks by preventing air contact. Aquapel is toxic, so minimize skin and inhalation exposure.

Load a second glass syringe with FC-40.

Put 27G needles threaded with 1.09mm O.D. tubing on both Aquapel and FC-40 syringes.

Fill devices with Aquapel: Insert the Aquapel syringe tubing into the oil port and carefully fill the device. As the Aquapel enters the device, the devices become more or less invisible. Note that faulty devices don't flow properly – if this is the case then the occluded channels will stay white - if that happens, cross out the device with a felt pen so it will not be used for Drop Seq experiments. The inlets and outlet ports will still be easy to see, so there should be no difficulty finding them for tube insertion. Fill each device in the column you are working on (treating groups of 8 or 10 devices at a time allows enough residence time for the Aquapel while streamlining the treatment process).

Expel Aquapel: Insert tubing that is attached to house air or a condensed air can into the oil port and blow air, holding an absorbent wipe over the other 3 device ports to prevent Aquapel from spraying. Expel Aquapel from all the devices in the column being treated, then proceed to next step.

Flush Devices with FC-40: Insert the FC-40 syringe tubing into the oil port. Insert 3 additional pieces of tubing in the remaining 3 ports and place the other ends into a 50 mL waste-collection tube. Flow enough FC-40 through the device to expel residual Aquapel.

Expel FC-40: Replace the FC-40 tube in the first port with the air tube. Blow the oil out, catching the waste in the 50 mL tube. Now remove the waste tube from port 2, replace it with the air tube, and again blow out the oil. Ditto for port 3. Repeat with remaining devices.

Repeat entire procedure with the next column of devices.

When all devices are treated, bake the chip at 65°C for 20 minutes.