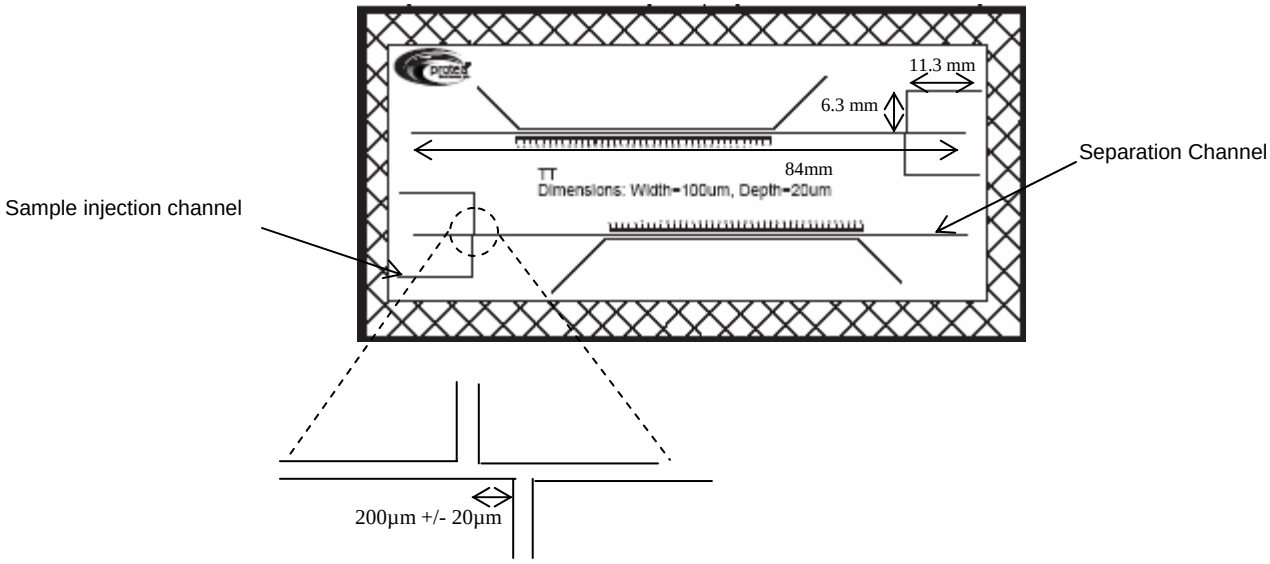
Instruction Sheet for Standard Microfluidic TT chip



Materials provided:

Two pre-assembled Standard Microfluidic Double T (TT) Chips.

Design:

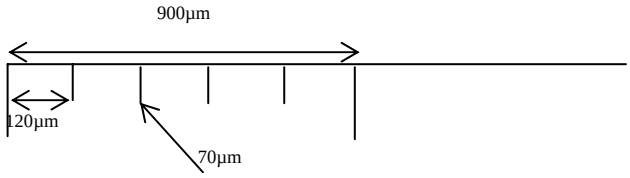
The TT standard chip comes with two “T” structured Microfluidic channels and is commonly used for Capillary Electrophoresis. The length of the separation and sample injection channels are 84mm and 17.6mm respectively, with the sample plug injection width being 200µm +/- 20µm.

Features:

Additional features of the design include a concentration gradient channel and a ruler. The concentration gradient channel may be used to flow through a known concentration of sample which then may be compared with that in the channel of

www.proteabio.com
For Tech Support: (304) 680-2660
smiti.bhattacharya@proteabio.com

the TT structure to provide a visual comparison. A ruler is also included alongside the channel to ease distance related measurements. The specifications of the markings on the ruler are as follows:



Use:

The following steps are to enable for proper Chip use and care:

Bonding Nanoports and Microtight fittings

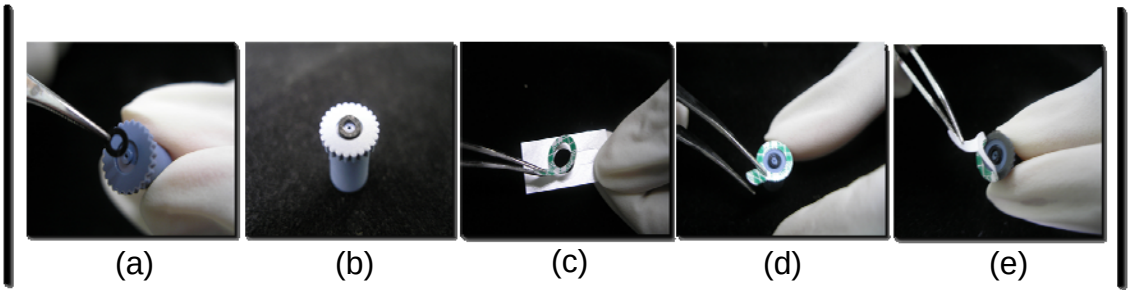
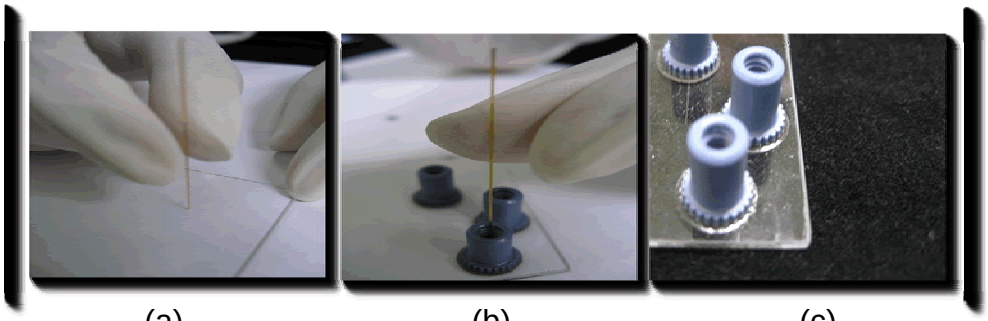


Figure 1. Coned port installation steps: (a) Insert gasket seal into recess at bottom of coned port (b) Press down to make sure gasket is snug in place (c) Remove one side of adhesive backing with tweezers (d) Center and place open adhesive side of ring to bottom of port (e) Ensure good contact has been made with base of port by applying light pressure around circumference and remove adhesive backing

www.proteabio.com
For Tech Support: (304) 680-2660
smiti.bhattacharya@proteabio.com



(a) (b) (c)

Figure 2. Aligning coned port on Chip: (a) Insert capillary into machined hole (b) Thread prepared coned port through capillary and center (c) Remove capillary and apply pressure to port making sure it doesn't get displaced

1. Prepare the surfaces to be bonded by cleaning both with isopropyl alcohol.
2. Invert the Coned Port and insert the gasket seal into the recess in the bottom of the port.
3. Remove one side of the adhesive backing with a pair of tweezers.
4. Center and place the open adhesive slide of the ring to the bottom of the Port. Ensure good contact has been made to the bottom of the Port by applying a light pressure around the circumference with a blunt surface.
5. Remove the other side of the adhesive backing.
6. Place a length of 360µm OD fused silica capillary in the drilled hole and thread the Coned Port through the capillary being careful not to break the capillary in the hole and center the port around the access hole on the substrate.
7. Gently remove the fused silica capillary from the Coned port and apply light pressure to ensure full contact between adhesive and both surfaces.
8. Cure the adhesive by baking in an oven at standard temperatures. For baking conditions, refer to Table 1 below.

Bonding a Nanoport reservoir

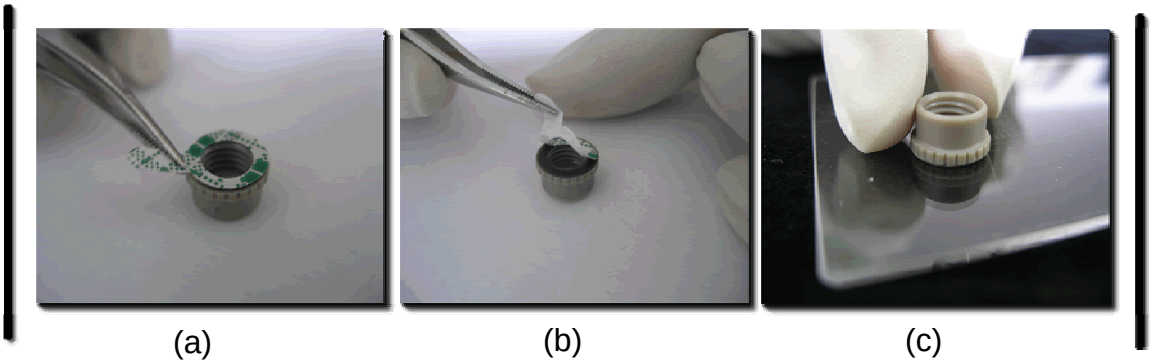


Figure 3. Nanoport installation steps: (a) Invert Nanoport and center adhesive upon removing one side of adhesive backing (b) Ensure good contact has been made with base of port by applying light pressure around circumference and remove adhesive backing (c) Place Nanoport on substrate around the access hole applying light pressure

1. Repeat steps 1 through 5 as directed in section 3a above.
2. Position the Nanoport around the access hole on the substrate and ensure contact is made between adhesive, substrate and port by applying a light pressure.
3. Cure the adhesive by baking in an oven at standard temperatures. For baking conditions, refer to Table 1 below.

Curing the adhesive on the ports

Apply a pressure on the ports by placing a weight (for example a block of metal of weight 0.5Kg) on them. Place this arrangement in an oven. The following chart details the amount of time necessary to develop a complete bond at various temperatures.

Cure Temperature	Cure Time
$250^{o}F$ /$121^{o}C$	95 minutes
$275^{o}F$ /$135^{o}C$	42 minutes
$300^{o}F$ /$149^{o}C$	20 minutes
$325^{o}F$ /$163^{o}C$	12 minutes
$350^{o}F$ /$177^{o}C$	6 minutes

Table 1. Time required to bond ports at different

*Allow for chip/s to cool down before removing from oven and testing.

www.proteabio.com
For Tech Support: (304) 680-2660
smiti.bhattacharya@proteabio.com

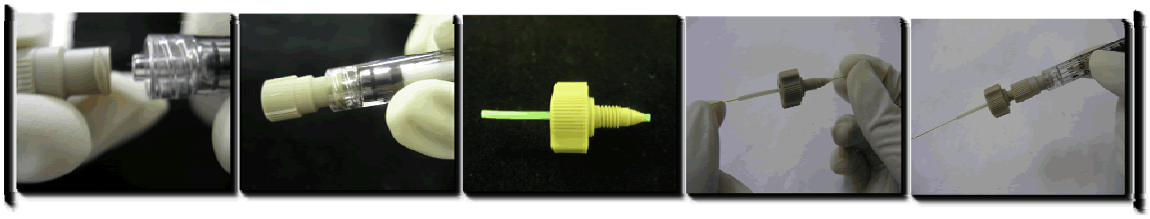
Filtering solutions prior to use

Figure 4. Filtering solutions: To reduce the possibility of clogging in microchannels, filter solutions prior to running them through the Microfluidic device

1. Prior to filling the syringe with solution, draw some air into the syringe, allowing the air to flush any solution retained in the filter[a].
2. Fill the syringe with the solution that is to be filtered.
3. Peel off the back of the package housing the filter. Holding the package in one hand and the syringe in the other, secure the filled syringe without excessive force to the filter device with a twisting motion.
4. Apply a gentle thumb pressure to begin filteration. A gentle pressure will allow a maximum throughput, whereas using higher pressures may rupture the filter.

[a]These filters are for single use only.

Capillary to Syringe



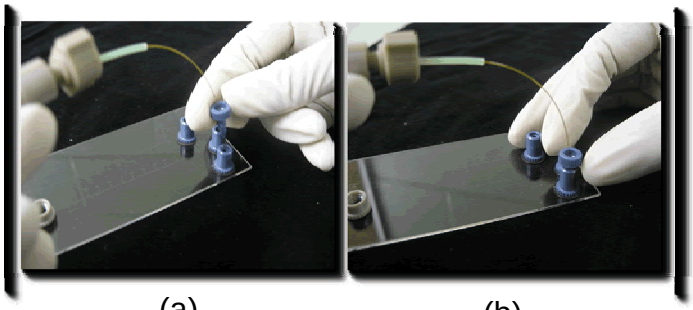
(a) (b) (c) (d) (e)

Figure 7. Interfacing a capillary with a syringe: (a)(b) Attach female end of adapter to syringe by rotating applying slight pressure (c) Pass green Teflon sleeve through fingertight nut (d) Pass fused silica capillary through Teflon sleeve (e) Attach fingertight nut onto adapter by rotating and applying slight pressure

www.proteabio.com
For Tech Support: (304) 680-2660
smiti.bhattacharya@proteabio.com

1. Attach the female end of adapter to the end of the syringe by rotating with a slight pressure.
2. Pass the green Teflon tubing sleeve through the fingertight nut.
3. Thread the silica capillary through the Teflon sleeve.
4. Attach the fingertight nut onto the Female Luer to Female adapter on the syringe by rotating and applying a slight pressure.

Interfacing Fused Silica capillary with the microchannel



(a) (b)

Figure 8. Interfacing capillary with microchannel (a) Thread free end of capillary from syringe into the 6-32 nut for coned port (b) Rotate nut into coned port until tight

1. Take the free end of the fused silica capillary from the setup (Figure 8) and thread it into a 6-32 nut for a 360µm OD capillary.
2. Rotate the nut into the coned port until fairly tight seal has been made.

Channel cleaning solution

Protea recommends cleaning your chip prior to its first use and between tests. This can be easily done by rinsing with a 0.1mM Sodium Hydroxide solution for 10 minutes, followed by a 5 minute rinse with de-ionized water. Then rinse with the appropriate CE buffer for 1 minute.

Chip is now ready for your experiments.

www.proteabio.com
For Tech Support: (304) 680-2660
smiti.bhattacharya@proteabio.com

Notes:

Sealing an access hole in a microfluidic device

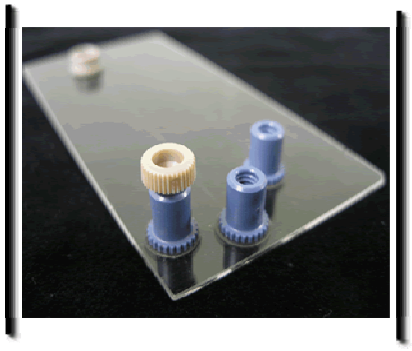


Figure 9. Sealing an access hole: Seal any particular access hole in a microfluidic device with a 6-32 Microtight plug

In the course of running experiments using your Microfluidic device, it often becomes necessary to seal certain access holes in the design. This may be done using a 6-32 Microtight plug as shown in Figure 9. As shown, the access hole is sealed simply by replacing the 6-32, 360µm nut with the Microtight plug.

Ordering Fittings or re-ordering Chips:

- By Phone: 1.877.776.8321
- By Fax: 304.292.7101
- Online: www.proteabio.com / orders@proteabio.com
- By Mail:
  Protea Biosciences, Inc.
  955 Hartman Run Road #210
  Morgantown, WV 26507