



Instruction

Installing a new Eppendorf CellTram 4 Oil and mounting Eppendorf TransferTips

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These instructions describe the installation of a CellTram 4 Oil for use with TransferTips ES or TransferTips ICSI.

Part 1: How to set up and fill a new manual microinjector CellTram 4 Oil

Part 2: Detailed instructions about mounting a microcapillary (TransferTips) onto the CellTram 4 Oil

By following these instructions you should be able to reproducibly install a CellTram 4 Oil and mount TransferTips. With practice, you may discover a better way to set up the system for your specific application.

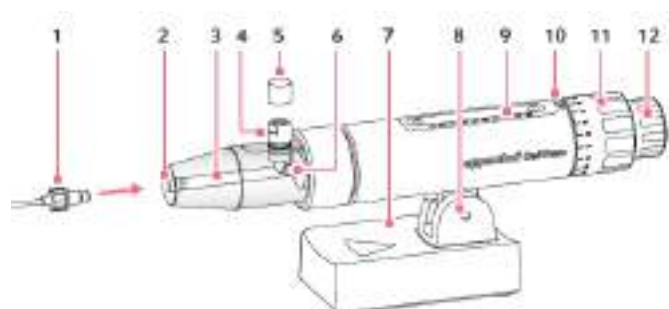


Fig. 1: CellTram 4 Oil

- | | |
|---------------------------|--|
| 1 Injection tube Oil | 8 Joint |
| 2 Port for injection tube | 9 Scale for piston position |
| 3 Cylinder | 10 Rotation direction indicator
IN – aspirate
OUT – dispense |
| 4 Filling valve | 11 Coarse drive
Rotary knob with scale for coarse drive |
| 5 Dust cap | 12 Fine drive
Rotary knob for fine drive |
| 6 Piston | |
| 7 Device foot | |

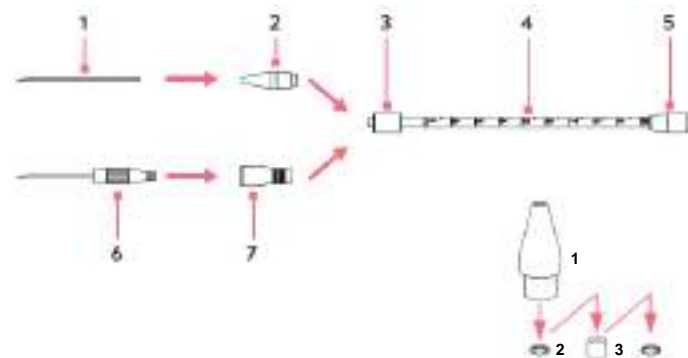


Fig. 2a: Capillary holder 4

- | | |
|---|---|
| 1 Capillary
(not included in the delivery package) | 5 Port for injection tube |
| 2 Grip head 4
Example with size Ø | 6 Ferretips
(not included in the delivery package) |
| 3 Knurled screw | 7 Adapter for Ferretips |
| 4 Capillary holder
Width scale for positioning | |

Fig. 2b: Grip head 4 system

- | |
|-------------------|
| 1 Grip head 4 |
| 2 O-ring (2x) |
| 3 Distance sleeve |

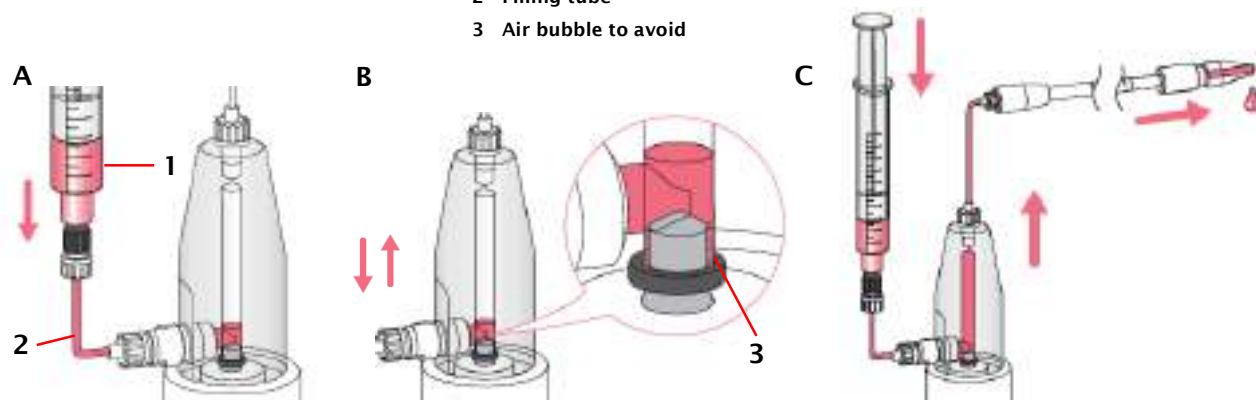
Part I - Setting up and filling the CellTram 4 Oil

Please be patient, it only takes about 5 min to properly fill a CellTram 4 Oil. Trying to hurry will lead to air bubbles in the system, forcing you to start over. **When properly filled there should be no air bubbles anywhere in the system except for one air pocket in the front of the TransferTip (see Part II).** Any other air bubbles make injections difficult to control.

1. Remove your new CellTram 4 Oil from the package. Turn the coarse control knob (see Fig.1: 11) counter-clockwise to move the piston backwards to piston position 10 (see Fig.1: 9). In this position the metal piston within clear acrylic oil chamber stops at the rear of the chamber (see Fig. 1: 3,6). You will feel resistance). Do not over-turn.

Fig. 3: Oil filling valve system

- 1 Filling syringe
- 2 Filling tube
- 3 Air bubble to avoid



2. Screw one end of the injection tube into the front of the CellTram 4 Oil (see Fig.1: 2) and attach the other end to the rear of the Capillary holder 4 (see Fig.2a: 5). Prepare the grip head 4 with two o-rings and the distance sleeve as shown in Fig. 2b. Mount the grip head 4 system onto the front end of Capillary holder 4 (see Fig.2a: 3) **but make sure not to screw it too tightly, let it loosen about one third of a turn.**
3. Attach the CellTram 4 Oil filling tube to a the filling syringe (10 mL-syringe with Luer lock). Aspirate about 1.0 mL of air into the syringe, and then slowly draw 3.5 to 4.0 mL of oil into the syringe from the bottle provided. Make sure you draw the oil in slowly; it should take about a minute to load the syringe. If you draw the oil in too quickly gas bubbles will come out of solution and you will need to start over. Once the syringe is loaded, hold it upright and tap it so that any air bubbles will float to the top. Blow all of the air out of the syringe and connected filling tube.
4. Remove the dust cap from the filling valve (see Fig.1: 4, 5). Screw the threaded end of the filling tube into the filling valve (see Fig.3a) on the CellTram 4 Oil. Fill the oil chamber about half way with oil. Leave the syringe attached to the filling valve and hold the CellTram 4 Oil vertically with the oil chamber pointing toward the ceiling. You can rest the base of the CellTram 4 Oil on the edge of a counter, but make sure you can still turn the knobs.
5. The metal piston needs to be primed with oil. Using the coarse knob move the piston forward about 1.0 cm and then backward until it stops (see Fig. 3b). **Do this 4 to 5 times.** Because the oil chamber has a large bubble of air in it you can turn the coarse knob very quickly at this step and the oil will not degas. Each time you change directions watch the area around the head of the piston. Small air bubbles will flow into the oil chamber (see Fig. 3b: 3). When no more air bubbles come out, pull the piston all the way back, then rapidly twist the coarse knob one rotation forward and back several times. If no more air bubbles appear you are ready to fill the CellTram 4 Oil. The piston only needs to be primed the first time the CellTram 4 Oil is used.

6. Hold the CellTram 4 Oil vertically in one hand and tap it so that the small air bubbles float to the top of the oil chamber. Pick up the filling syringe with the other hand and slowly fill the oil chamber. Make sure you blow all of the air out of the oil chamber and that the oil has filled several centimeters into the injection tube. Set the CellTram 4 Oil down on its base and continue to fill it until oil drips out of the front of the capillary grip head 4 (see Fig. 3c). There should be no air bubbles in the system.
7. To avoid a siphon effect, set the Capillary holder 4 on something so that the end of the capillary grip head 4 is level with the filling valve. Carefully unscrew the filling tube from the filling valve. By removing the threat of the filling tube the self-closing filling valve is closed. Don't forget to place back the dust cap onto the filling valve.
8. Return any unused oil to the bottle, put the syringe with the filling tube attached into a zip-lock bag and clean up. You are now ready to attach a TransferTip and begin working. Now that the system is sealed, be careful not to turn the coarse knob faster than about one full rotation every 3 seconds.

Once the CellTram 4 Oil is filled with oil and sealed, turning the coarse rotary knob too quickly counter-clockwise will result in too strong of a vacuum and may degas the oil - gas may be pulled out of solution forming bubbles in the oil chamber or in the injection/pressure tube. Too high pressure can fire a microcapillary out of the capillary holder into the room and risks injury. When the system is sealed, be careful to always turn the coarse knob slowly, no faster than about one full rotation every 3 sec. The fine control knob (see Fig.1: 12) is normally used for injections and turning this quickly will not cause any problems.

Changing microcapillaries:

Each time you remove a TransferTip you create air bubbles in the capillary grip head 4 and the Capillary holder 4. You need to remove these air bubbles out of the oil phase (there are usually 2 or 3 small bubbles) before you install a new TransferTip.

Refilling the system with oil:

As you work with the system, you will gradually lose oil each time you change tips. To refill the system, repeat the above instructions skipping step 5).

Not recommended: *In case you prefer to aspirate the oil in through the front of the system: **Loose the grip head 4 approx. one third to half of a turn to remove any capillary from the grip head 4. Bleed any air bubbles out of the capillary holder and the grip head by turning the knob on the CellTram 4 Oil clockwise. When all air bubbles have been removed, submerge the grip head in the bottle of oil up to the knurled part of the capillary holder. Slowly fill the system with oil by using the coarse rotary knob. Wipe off the grip head and capillary holder, and you are ready to continue working.***

Part II - Mounting the Eppendorf TransferTips onto CellTram 4 Oil

For good injection performance it is important to learn how to properly mount a microcapillary onto the microinjector. If it is not properly mounted, the system can become unstable, difficult to control, or the microcapillary can become filled with bubbles making it impossible to use.

There are several ways how to work with microcapillaries using oil based injectors.

The following is a proposal for a technique for mounting the microcapillaries:

There are several ways how to work with microcapillaries using oil based injectors.

The following is a proposal for a technique for mounting the microcapillaries which keeps an air pocket between the oil in the base of the TransferTip and the media in the front of the which should be kept along the work process.

It is also advisable that you not allow the media and oil to meet, and that you avoid drawing media into an area of the capillary where oil has already been. Oil coats the inside of the capillary as it passes, so if you pull media through a section of the capillary where oil has passed, droplets of oil coalesce off of the capillary walls. These oil droplets stick to the inside of the capillary and prevent you from drawing cells inside. They will force you to use higher pressures and vacuums. This makes the injection process very difficult to control. Even if you are able to blow the oil droplets out, they just reform and the liquid inside the tip becomes increasingly compartmentalized with layers of oil and media. When this happens the tip will eventually need to be discarded. The method of mounting a TransferTip described below helps you avoid this unpleasant situation.

When you are learning to use CellTram 4 Oil have extra TransferTips available so you can change microcapillaries frequently. You will quickly improve and in a few days you should find that you consistently use fewer tips.

1. To install a new TransferTip, you might leave the Capillary holder 4 mounted onto the angle head of the micromanipulator motors. Just use the “home function” of the Eppendorf micromanipulators to drive the used microcapillary out of the working position under the microscopic focus. Swing out the motor with the capillary holder to the front.
2. Remove the used capillary out of the grip head by loosen the grip head 4 system at the Capillary holder 4 (see Fig.2: 2,3) by one third to half of one turn and discard the used capillary – they are not reusable.
3. Fix with your index finger the slightly loosened grip head against the capillary holder to avoid any air bubbles that may appear between the grip head and capillary holder and then turn the fine or coarse control knob (see Fig.1: 11,12) on the CellTram 4 Oil to purge any unseen air bubbles within the capillary holder or grip head. Removing a capillary out of the grip head usually leaves also two or three bubbles hidden in the O-rings and distance sleeve of the grip head (see Fig. 2b).

4. When the bubbles have been removed out of the capillary holder and the grip head, expel a small amount of oil from the front of the capillary grip head (have paper towels or Kimwipes ready) by turning the coarse rotary knob.
5. Still **with the index finger fixing the grip head** insert the new TransferTip into the grip head and slide the back end of the microcapillary completely through the O-rings in one movement. If you are careful, you will feel the back end of the TransferTip make contact with the front end of the capillary holder. Grasping the knurled fitting on the front end of the Capillary holder 4 in one hand, twist the capillary grip head clockwise with the other hand to tighten. The O-rings should now hold the TransferTip securely; you can test this by tugging gently on the glass capillary. If it slips forwards easily then the grip head needs to be tightened more. If the grip head cannot be tightened securely, check to make sure that you are using the correct grip head for the diameter of glass capillary used, and make sure that the O-rings are properly installed.
6. Once the TransferTip is held snugly in the grip head 4 system, it is advisable to keep the oil meniscus within the tubing of of the capillary near the capillary grip head. In case your capillary holder is already mounted onto the micro-manipulator swing back the capillary holder to the centered position. In case the capillary holder was not mounted onto the manipulator, fix the capillary holder to your micromanipulator and get your cells ready to work. Check how far down the shaft of the TransferTip the oil comes.

Suggested technique for loading of ES cell or ICSI TransferTip capillaries using CellTram 4 Oil

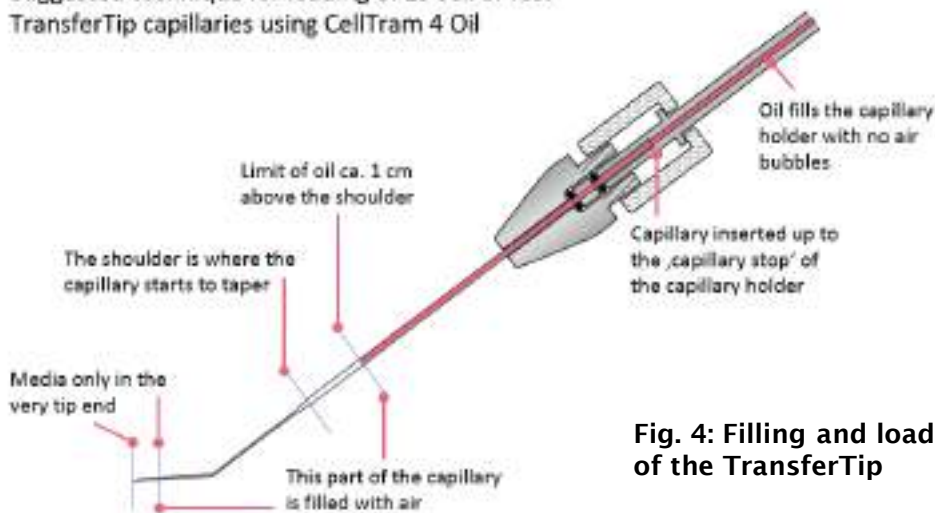


Fig. 4: Filling and loading of the TransferTip

7. Before lowering the microcapillary into the dish, look at your TransferTip and locate the meniscus between the oil and the air. Carefully turn the fine control knob on the CellTram 4 Oil clockwise to move the meniscus down the shaft of the TransferTip until it is approximately 1.0 cm above the place where the TransferTip begins to taper. This is called the shoulder (see Fig. 4). The system works most reproducibly if you leave the oil about 1.0 cm above the shoulder of the capillary. Once you have moved the oil to that point, check it to make sure that it does not drift further down.

8. When you are satisfied that the air/oil interface is stable, bring your microcapillary into position above the stage. It may be easier to find the capillary if you lower it over the edge of the media drop, and then move it to the center. Gently rotate the capillary holder in its fitting to properly align the TransferTip before lowering it. Just before the capillary touches the surface of the oil overlay, give the fine knob on the CellTram 4 Oil a half turn clockwise. This is to put a slight positive pressure on the TransferTip to prevent it from front-filling with oil as you pass through the oil overlay.
9. Move the microcapillary quickly through the oil into the media. Air may be slowly bubbling out of the end of the TransferTip or media may be filling the microcapillary through capillary forces. Carefully and gently adjust the flow of the CellTram 4 Oil until it reaches equilibrium. Then very carefully bring the air/media interface towards the end of the TransferTip so that there is only a
10. Learn how to control the air/media meniscus. Do not let the media interface go farther than 10 to 20 μm up the shaft from the capillary until you are ready to pick up cells. Try not to allow any air to blow out of the capillary. Gently move the meniscus back and forth. You should find that the media/air meniscus is very stable and can be easily controlled with fine control knob on the CellTram 4 Oil. When you stop turning the knob, the meniscus should immediately stop moving.
11. **Working with TransferTip ES for ES cell transfer.** Before you pick up your cells draw in only enough media to push your cells back out when you are ready to inject. For ES cell work, don't pick up much extra media, just pick up two or three extra stem cells which you will keep in the capillary and use to push the others out. See the illustration to the right. Try not to let the media/air interface leave the field of view in your microscope.

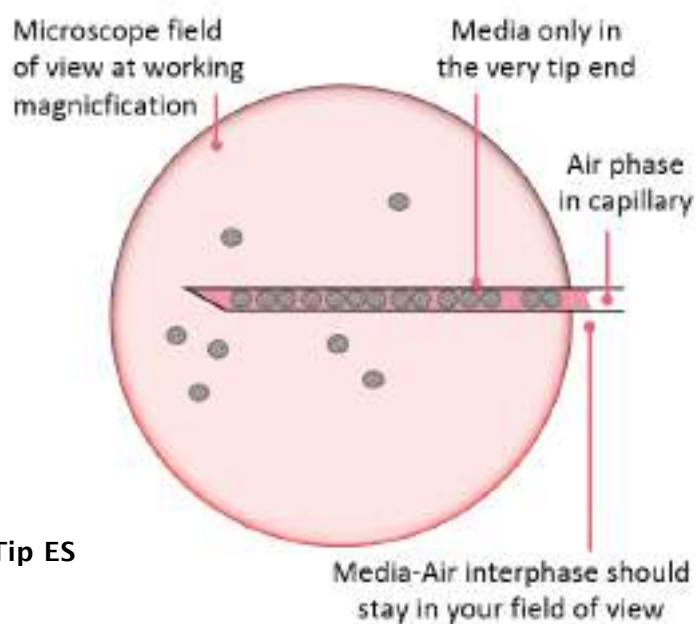


Fig. 5: Filling the TransferTip ES with CellTram 4 Oil

11. Working with TransferTip ICSI for injection of sperms: When you are ready to pick up a sperm, draw the air/PVP meniscus up the shaft of the TransferTip about 3 sperm lengths (including the tail) from the tip. ICSI needles are used less and so you will not have too much trouble if you pull in a little extra PVP. See illustration to the right. Try not to let the air/PVP interface leave the field of view in your microscope, although this may be necessary when rupturing the plasma membrane.

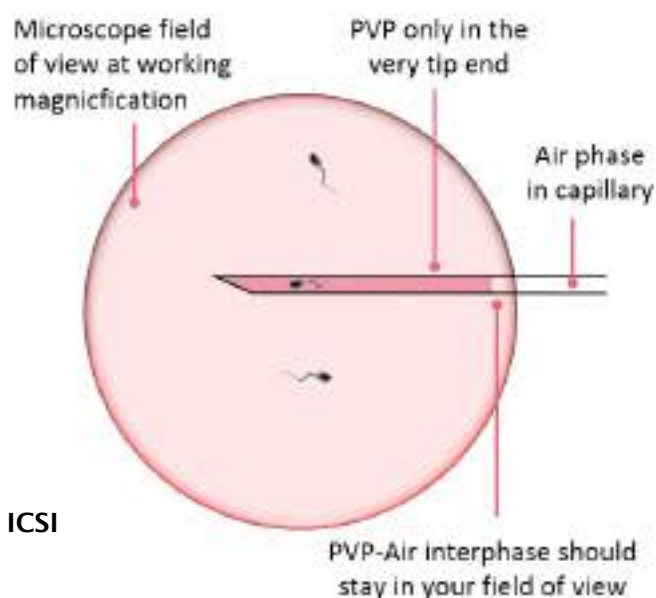


Fig. 6: Filling the TransferTip ICSI with CellTram 4 Oil

Setting up the CellTram 4 Oil and properly mounting the tips becomes easier with practice. Working in routine it will take less than a week to get used to this system (assuming one or two hours practice per day). After that time things get much easier.

Following these instructions you should be able to reproducibly install CellTram 4 Oil and mount TransferTips so they will work each time.