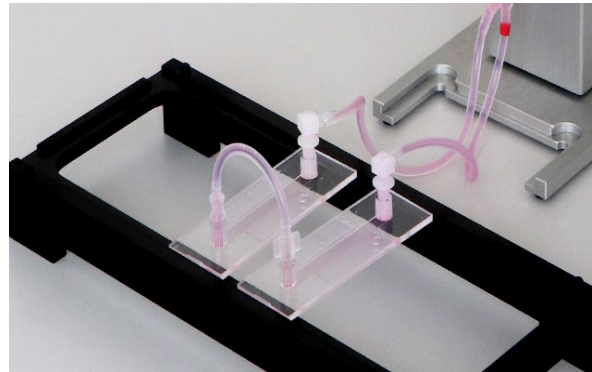


## Serial Connection of Luer-Slides for Flow Experiments

The ibidi Pump System is specially designed for long-term experiments of cells under perfusion. The standard setup connects one slide to one Perfusion Set. In some cases, however, it might be beneficial to increase the number of slides (i.e., cells), for example, when planning numerous stainings or if the cell number is not sufficient for the subsequent applications (e.g., Western Blot, see [Application Note 38: Western Blot Analysis with Cell Samples Grown in Channel- \$\mu\$ -Slides \(PDF\)](#)).



This Application Note is a step-by-step protocol showing how to create a serial connection between two  $\mu$ -Slide I<sup>0.6</sup> Luer channels. The protocol only specifies the procedure for two slides but can be applied to a larger number of slides correspondingly.

**Important Notes:** Serially connected slides experience the very same flow rate and hence the very same shear stress.

Since this is not the case in parallelly connected slides, we absolutely recommend connecting the slides in series, but not in parallel!

We recommend connecting not more than 4–5  $\mu$ -Slides in the described way.

### Related Documents

[Application Note 3: Cell Culture in ibidi Channel Slides \(PDF\)](#)

[Application Note 13: Endothelial Cells Under Perfusion \(PDF\)](#)

[Application Note 31: Serial Connection of  \$\mu\$ -Slide VI<sup>0.4</sup> Channels for Flow Experiments \(PDF\)](#)

[Instructions  \$\mu\$ -Slide I Luer Family \(PDF\)](#)

[Instructions ibidi Pump System \(PDF\)](#)

### Material

- 2  $\mu$ -Slides I<sup>0.6</sup> Luer, ibiTreat (80186, ibidi)
- Endothelial cells (e.g., HUVECs)
- Endothelial Cell Growth Medium (e.g., PromoCell)
- $\mu$ -Slide Rack (80003, ibidi)
- ibidi Pump System (10902, ibidi, please see the [ibidi Pump System Instructions](#) for details)
- Perfusion Set (e.g., RED, 15 cm, ID 1.6 mm, 10962, ibidi)
- Hose Clip (part of the ibidi Pump System)
- Serial Connector (10830, ibidi; for alternative assembly, see page 2)
- Sterile syringe (1 ml) with simple Luer adapter (various suppliers)
- Standard cell culture equipment (sterile working bench, cell detachment kit, culture flasks, pipets, tips, etc.)

Alternatively, assemble the Serial Connector using the following parts:

- Silicone Tubing 1.6 mm ID (10842, ibidi)
- Luer Connector Male (10824, ibidi)

Shorten the tubing to a length of 6 cm. Then plug one plastic connector into each end of the tubing. This results in a connector tubing with two free male Luer adapters at the ends to connect channels between slides. Finally, sterilize the connector with ethanol or by autoclaving.



**Figure 1:** Example of an assembled Serial Connector.

## 1. Preparations

**Important Note:** Equilibrate all material, such as the  $\mu$ -Slides, the cell culture medium, the Serial Connectors, and the Perfusion Set **overnight** in the incubator at 37°C and 5% CO<sub>2</sub>. This step is essential to prevent air bubbles from emerging over time during the experiment.

### 1.1. Cell Seeding

Perform all the following protocol steps under sterile working conditions!

First, unpack the  $\mu$ -Slides and place them on a  $\mu$ -Slide Rack or petri dish to prevent the slides from cooling down while working on the cold metal surface of the laminar flow hood. Next, cover the Luer adapters with the caps that come with the  $\mu$ -Slide, while preparing the cell suspension.

- Prepare a cell suspension of  $1.67 \times 10^6$  cells/ml to achieve a final cell number of  $1 \times 10^5$  cells/cm<sup>2</sup> or  $2.5 \times 10^5$  cells/ $\mu$ -Slide.
- Remove all caps from the reservoirs.
- Fill the channels with 150  $\mu$ l cell suspension. Only the inside of the channel is filled (Fig. 2A).
- Put the caps on the Luer adapters and incubate the slides at 37°C and 5% CO<sub>2</sub> for approximately one hour, allowing the cells to attach.
- After cell attachment, fill each reservoir with 60  $\mu$ l cell-free medium and close them with the caps.
- The cells/slides are now prepared for serial connection. Depending on cell growth and metabolism, the slides can be stored in the incubator for up to 24 h. For most experiments, the cells should form an optically confluent monolayer.

For a detailed description of the cell seeding procedure, please refer to the [μ-Slide I Luer Instructions, Application Note 3, "Cell Culture in ibidi Channel Slides"](#), and [Application Note 13, "Endothelial Cell Culture Under Perfusion"](#).

### 1.2. ibidi Pump Setup

Set up the ibidi Pump as described in the [ibidi Pump System Instructions](#) and [Application Note 13, "Endothelial Cell Culture Under Perfusion"](#). Fill the Perfusion Set with its total working volume, adding media to each reservoir to create a 5 ml equilibrated medium each reservoir. Then remove the air bubbles by running the ibidi Pump with a middle range pressure (around 50 mbar). While running the program, gently tap on the tubes and the adapters with your fingers to remove the air bubbles. The level of both reservoirs should be re-equilibrated to 5 ml after all bubbles are removed from the tubes.

**Important Note:** It is crucial to remove all air bubbles before connecting the slides to the Fluidic Unit! Any gas remaining in the system can influence the flow rate and, in the worst case, stop the flow or flush the cells away.

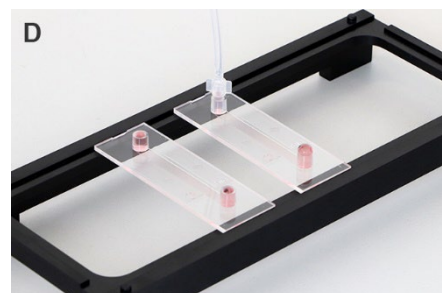
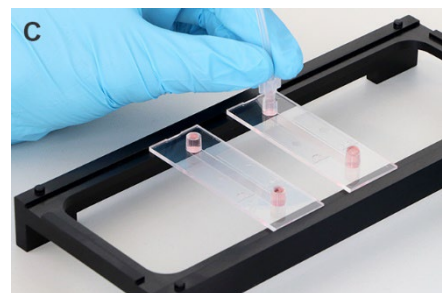
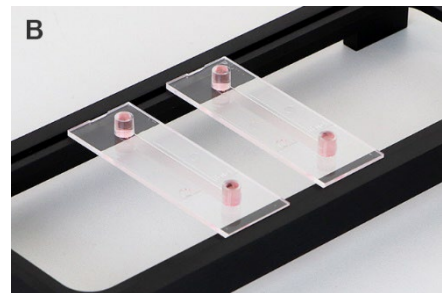
## 2. Connecting the $\mu$ -Slides I Luer<sup>0.6</sup>

After cell seeding and attachment, follow these steps to connect 2  $\mu$ -Slides I Luer<sup>0.6</sup> serially. Perform these steps as fast as possible to reduce cellular stress caused by temperature fluctuations or too much agitation, which would lead to cell detachment.

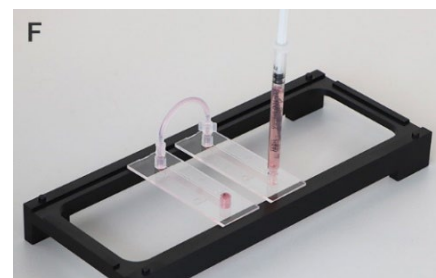
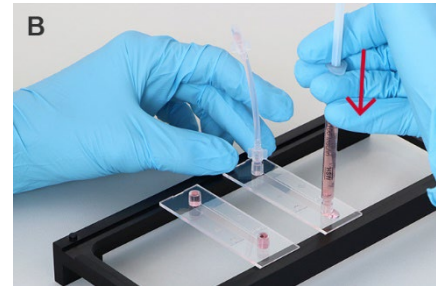
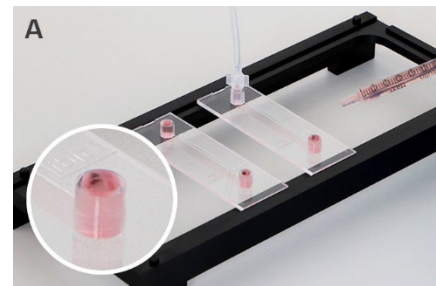
Use caution when connecting the male Luer adapter to the female Luer port to help reduce trapping air bubbles within the channel.

We recommend placing the  $\mu$ -Slides on a  $\mu$ -Slide Rack or petri dish to prevent the slide and the cells from cooling down while working on the cold metal surface of the laminar flow hood.

- Put the  $\mu$ -Slide Rack, the syringe, the equilibrated medium, and the equilibrated Serial Connector into the laminar flow hood. Place the  $\mu$ -Slides containing the cells on the  $\mu$ -Slide Rack (Fig. 2A).
- Fill all  $\mu$ -Slide reservoirs with cell-free medium until the top. There should be a small hump of liquid (Fig. 2B).
- Plug one male Luer adapter of the Serial Connector into one of the female Luer adapters of one  $\mu$ -Slide (Fig. 2C). Twist the adapter while tightly pressing it into the Luer port. Wipe off any overspilled medium using a paper towel.
- The Serial Connector is now connected to the first  $\mu$ -Slide (Fig 2D).



- Fill the syringe with at least 1 ml of prewarmed medium. Reverse the syringe and push out the surplus air. There should be a small hump of medium (Fig. 3A).
- Connect the syringe to the free Luer adapter of the first  $\mu$ -Slide. Make sure no air bubbles are trapped inside (Fig. 3B). Wipe away any spillover with a paper towel.
- Very carefully, push the medium through the channel of the  $\mu$ -Slide (Fig. 3B) until the Serial Connector tubing is filled entirely with medium and you see a small hump of liquid (Fig 3C).
- Plug the male Luer adapter of the Serial Connector into the adjacent female Luer adapter of the second  $\mu$ -Slide (Fig. 3D–E).
- The slide is now ready for connection to the Perfusion Set (Fig 3F).
- In case you want to connect more than two slides, keep the syringe connected to the first slide. Place a second connector tubing onto the free adapter of the second slide, push the medium through using the syringe, and continue as described.



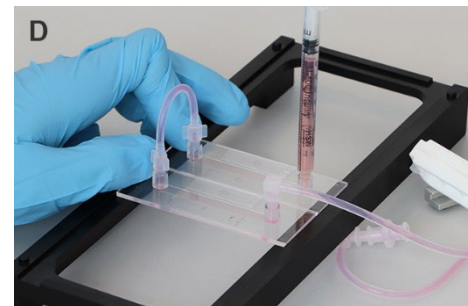
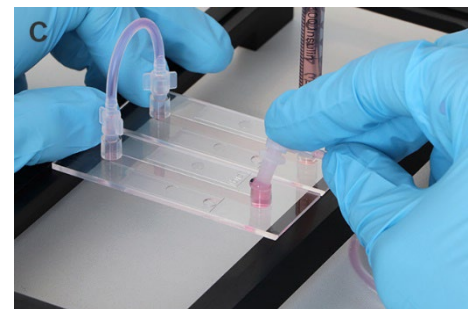
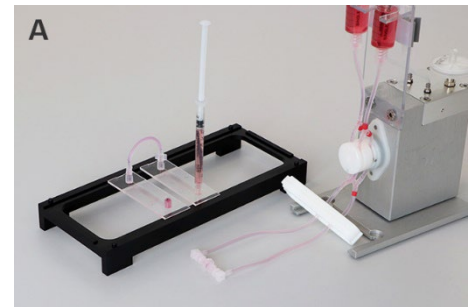


### 3. Connection to the Perfusion Set

For detailed instructions for connecting the slides to the Perfusion Set, please refer to [Application Note 13 “Endothelial Cell Culture Under Perfusion”](#), section 6 “Connecting the  $\mu$ -Slide I<sup>0.6</sup> Luer to the Perfusion Set.

Shortly, perform the following steps:

- Stop the flow of the ibidi Pump using the PumpControl software. Next, disconnect the Fluidic Unit (with the mounted Perfusion Set, filled with medium) from the ibidi Pump and place it in the laminar flow hood close to the serially connected  $\mu$ -Slides I<sup>0.6</sup> Luer. Pinch off the Perfusion Set tubing using the white plastic hose clip (Fig. 4A).
- Pull out one male Luer adapter from the middle connector by holding it upwards (Fig. 4B). Avoid trapping air bubbles inside.
- Tightly connect this male Luer adapter to the free female Luer port of the  $\mu$ -Slide (Fig. 4C).
- The Fluidic unit is now connected to one  $\mu$ -Slide (Fig. 4D).

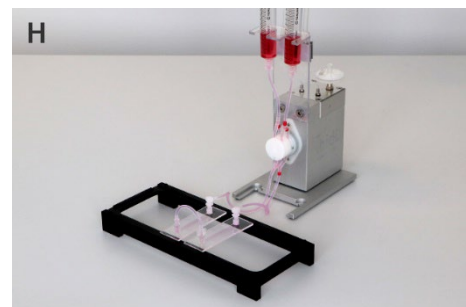
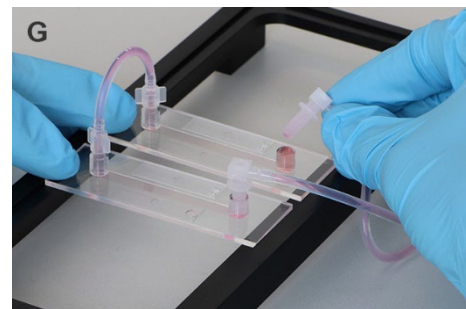
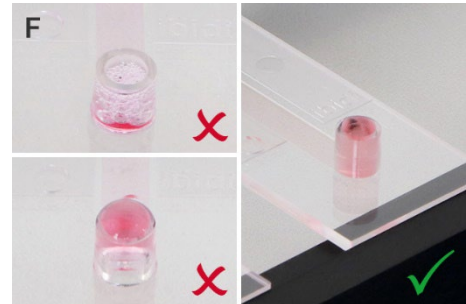
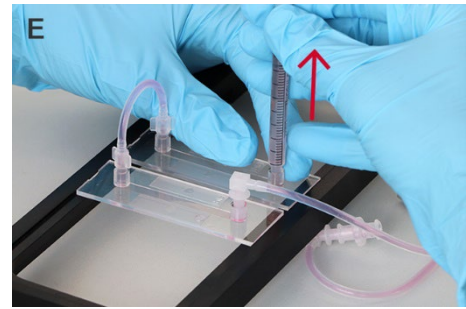


- Slowly remove the syringe from the other  $\mu$ -Slide (Fig. 4E).
- Check the Luer port for air bubbles (Fig. 4F) and, if necessary, remove them with a syringe or pipet.
- Disconnect the second male Luer adapter from the middle connector by holding it upwards (Fig. 4B) and connect it to the remaining female Luer port on the  $\mu$ -Slide (Fig. 4G).
- The serially connected  $\mu$ -Slides I<sup>0.6</sup> Luer are now connected to the Fluidic Unit. Wipe off any excessive medium with a paper towel and remove the white plastic clamp (Fig. 4H).

**Important Note:** We recommend checking the cells under the microscope before starting the perfusion experiment.

It is crucial that the cell layer is confluent, and the cells are well adherent when they are exposed to shear stress. If the cells become stressed by the connecting procedure, give them a few hours to recover in the incubator before starting the flow.

- Put the whole assembly into the incubator and connect the Fluidic Unit to the ibidi Pump System.
- Start the flow experiment.



## 4. Adapting the Flow Rate

The software does not provide a standardized setup of more than one  $\mu$ -Slide. Therefore, you will have to manually adapt the parameters of the ibidi Pump for the new demands.

Within the software, as a guideline, you can still select channel slide,  $\mu$ -Slide VI<sup>0.4</sup> and your Perfusion Set color setup. But the resulting flow rate (and hence also the shear stress) will be lower than in the single channel application.

Start a cycle with the desired shear stress and measure the flow rate by hand (see the ibidi Pump Instructions, page 40). Then open the recalibration dialogue and insert the calculated (given flow rate of the software) and the measured flow rate.