Please, read carefully
GETTING STARTED
BEFORE 1ST USE

Chitozen

PROTOCOL

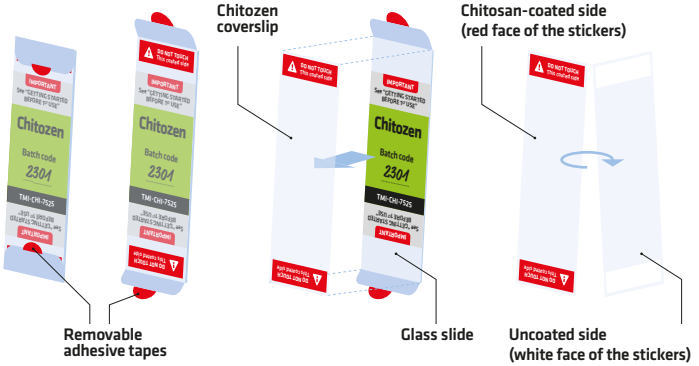
idylle

GETTING STARTED BEFORE 1ST USE

Chitozen is composed of two different slides:

- The Chitozen coverslip that has **one side coated with chitosan which is exposed to the open air.** It is very fragile.
- The glass protective slide that protects the Chitozen coverslip. It bears the green label with the batch code.

They are attached together with two red removable adhesive tapes.



Precaution of use

Always manipulate the coverslip by the edges and never touch the chitosan-coated surface.

Storage

Do not expose to high temperatures (>30°C) or brutal temperature variations. It can severely compromise the chitosan coating and thus adherence efficiency.

PROTOCOL

1. Preparing the material you need

CONSUMABLES

- ✔ Chitozen slide (included in the kit)

- ✔ Sticky-slide VI 0.4, 6 channels, cat n° 80608, ibidi (included in the kit)

REAGENTS

- ✔ LB medium*
- ✔ Milli-Q® water
- ❗ **Make sure you use fully deionized water as increased ionic strength can alter bacterial adhesion. If a Milli-Q water purification system is not available, we recommend using a molecular biology grade water.**

CELLS

- ✔ Escherichia coli or your favourite bacteria

HARDWARE (OPTIONAL)

- ✔ Microscopy rack, ibidi (provided upon request)

- ✔ Eppendorf ® Centrifuge 5430 & Rotor 5430R A-2-MTP
- ✔ Clamp and adapter for sticky slides, ibidi (provided upon request)

* Check our website for updated information on protocol recommandations for validated bacterial species.

2. Storing

- **Exposure to high temperatures (>30°C) or brutal temperature variations can severely compromise the chitosan coating and thus adherence efficiency.**
- Store at room temperature (20-25°C), in a dry place without direct sunlight.
- Unmounted Chitozen coverslips can be stored up to 12 months.
- Once mounted with a sticky-slide, Chitozen coverslips can be stored up to 2 months.

3. Assembling the elements

- If it is your first use of Chitozen, please go to "Getting started before 1st use"
- Remove the protection film of the sticky-slide.

- Place the sticky slide on the bench with the channels and the lid facing down.

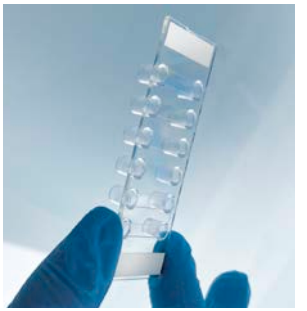
- Take the Chitozen coverslip from its box.
- **Always manipulate the coverslip by the edges and never touch the chitosan-coated surface.**

- Gently remove the two red adhesive tapes using tweezers.

- Remove the protective glass slide located below the Chitozen coverslip.

- Stick the chitosan-coated side to the sticky-slide.
- **Make sure the chitosan-coated side of the coverslip (where the red sticker is apparent) is facing your sticky-slide.**

- **You should spot the red stickers when you face the channels.**



- Gently press with your thumb along the channels from the center to the periphery to remove any air bubble that may have formed.
- **Do not press too firmly to avoid breaking the coverslip.**

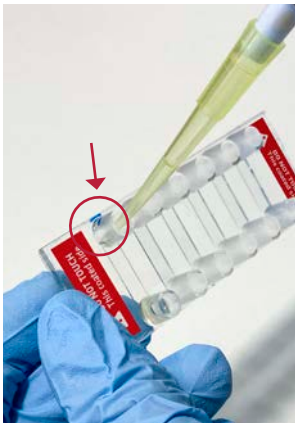
(Optional) Use the clamp for the sticky-slide to ensure that it is correctly sealed to the Chitozen coverslip.

- First put the slide in the appropriate adapter.

- You can press twice.

Gradually increase the pressure and do not press too hard to avoid breaking the coverslip.

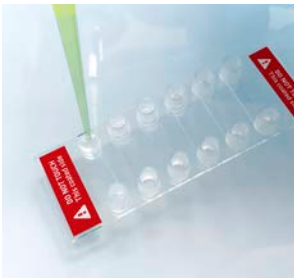
- Mark one well of a row as the entry well. By default, the other well of the row will be designated as the exit well.



Each channel is independent. It can be used the same day as other channels or not. If channels are still available, store your assembling at room temperature (20-25°C), in a dry place shielded from direct sunlight for up to 2 months.

4. Channel priming

- Add 150 µL of Milli-Q water in the entry well to rehydrate the chitosan.



- Wait for at least 15 minutes at room temperature.

The chitosan gel must not dry. Once the chitosan has been rehydrated, proceed up to the centrifugation step.

5. Sample loading

Experiments of the team of researchers have been performed with LB ½, prepared by mixing 50 mL of sterile LB and 50 mL of sterile Milli-Q water. Chitozen has been used by researchers with other culture medium such as complete LB and M9 media.*

- Measure the OD of your bacterial culture.
- Dilute the bacterial culture in LB ½ medium to reach a final OD of 0.01 in a final volume of 2 mL.

Do not prepare a final volume of bacteria suspension below 0.5 mL, that is not enough to perform an experiment.

You can test higher OD values for optimizations but avoid OD > 0.1.

- Remove 150 µL of Milli-Q water from the exit well of your channel, discard it. This volume is often smaller than 150 µL because of the rehydration of the chitosan.
- Rinse: Add 150 µL of bacterial suspension (OD=0.01) in the entry well and remove it immediately from the exit well. Repeat once.

Be careful not to introduce air bubbles in the channel.

- Finally, add 90 µL of bacterial suspension (OD=0.01) into the entry well and proceed immediately to the centrifugation.

It is possible to load up to 100 µL of bacterial suspension.

6. Bacterial adhesion to the Chitozen

Depending on the bacteria species, you can make bacteria adhere to the Chitozen thanks to a centrifugation or a passive sedimentation protocol.*

CENTRIFUGATION PROTOCOL

- Place the coverslip in the µ-Slide Microscopy Rack, on the plate rotor.

- Centrifuge at 850 g for 2:30 minutes at room temperature.

Media can flush out of the coverslip due to leftover air bubbles or abrupt movement when changing the centrifuge rotor. If your channel is empty after centrifugation, immediately load again 90 µL of your bacterial sample and centrifuge again.

* Check our website for updated information on protocol recommandations for validated bacterial species.

- Excess bacteria can be washed out by using a continuous flow as detailed in section 9.

PASSIVE SEDIMENTATION PROTOCOL

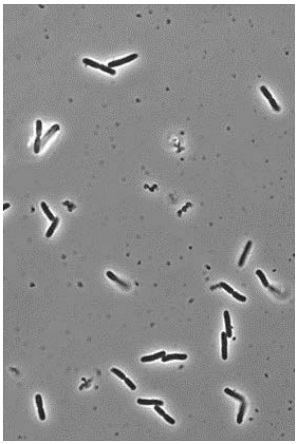
- After loading your bacterial suspension, leave the slide for 20 minutes at room temperature on the benchtop.
- Excess bacteria can be washed out by using a continuous flow as detailed in section 9.

7. Imaging

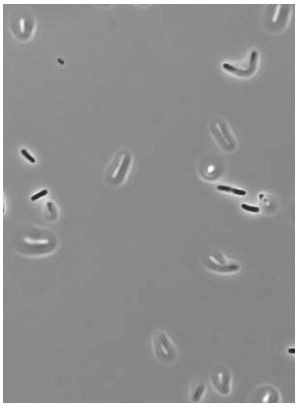
- Conduct your microscopy experiment immediately after assembly (e.g. 100X immersion oil) at room temperature.

8. Troubleshooting

- You may observe dust particles. They may be either due to the medium that interacts with the chitosan surface or to the quality of Milli-Q water.



- You may observe low adhesion after centrifugation. Adhesion efficiency may be altered due to the culture media or bacterial strains used, insufficient centrifugation or prolonged bacterial culture. Make sure the whole experiment was performed at room temperature and that the Chitozen slides were stored properly. It is possible to discard the floating bacteria by gently removing the medium from the exit well and load 90 µL of medium (without bacteria) in the entry well.



9. Going further: perfusing the system with flow

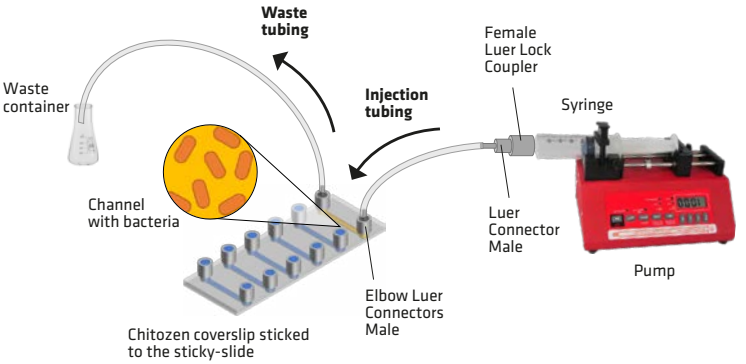
- Chitozen slides allow bacterial cells to be immobilized even under a flow. Assays were successfully performed with E. coli under a maximum flow of 5 mL/min. It is of course possible to adapt the protocol to your own experiments and requirements. For that, it is recommended to inject 100 µl of bacterial suspension instead of the 90 µl previously recommended into the channel before the centrifugation step. You will then avoid the formation of air bubbles when connecting the tubing and the channel.

MATERIAL

- Sterile syringe
- Tubing Thermo Scientific Nalgene, ref 8001-0102, metric 180 PVC tubing USP class VI, 1MMID 2MMOD 5MM wall, pack of 25M
- Flow ibidi accessories: tubing connectors (Luer connector male #10824, elbow Luer connector male #10802) and coupler (female Luer lock coupler #10823)

- Aladdin Syringe Pump WPI, AL1600

- LB ½ medium



PROTOCOL

Two kinds of tubing are connected to the Chitozen channels:

- 1 dedicated to injection: it is connected to an "elbow Luer connector male" at one side and to a "Luer connector male" on the other side. A syringe of 20 mL (or smaller if less volume is needed) is connected to this tubing on "the Luer connector male" side thanks to a "female Luer lock coupler" and filled with LB ½ medium.
- 1 dedicated to the waste elimination: it is connected to an "elbow Luer connector male" at one side and left free on the other side.
- After centrifugation of the Chitozen coverslip, loaded with bacteria, the tubing dedicated to injection is connected to the exit well of the channel.

Be careful not to introduce air bubbles.

- In the same way, the tubing dedicated to the waste elimination is connected to the entry well. This tubing allows the elimination of liquid so put it into a waste container.
- Install the syringe on the pump and setup the flow on the machine.

Be careful not to introduce air bubbles.

- Eventual non-adherent bacteria are eliminated thanks to a flow of 1,5 mL/min (up to 5 mL/min), with a 2-5 mL volume of appropriate medium (e.g. LB ½).
- Then a constant flow of 3 mL/h is applied to perfuse the channel with fresh medium and eliminate non-adherent bacteria.

CLEANING OF THE TUBING

- The tubing can be rinsed three times with 10mL of water, three times with 10mL of ethanol 96°, and finally three times with 10mL of water.
- Leave the tubing dry before the storage.

Check videos of protocol, examples
of results and much more on:
idylle-labs.com/chitozen-by-chitosan

A protocol designed in November 2021 and updated in April 2023 thanks to the feedback from the early users.

The pictures have been taken in April 2021 and December 2022 with Amandine Desorme and Laetitia My in the "Laboratoire de Chimie Bactérienne" lab.

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for his kind contribution.

idylle-labs.com
30, rue de Campo-Formio
Paris - France
+ 33 1 84 25 51 44
contact@idylle-labs.com

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