Chitozen

Questions & Answers

Q/ What is included in the Chitozen kit?

Two kit formats are available, with or without microchannel sticky slides, depending on your desired applications. The sticky slides will allow you to work in a closed system in static or dynamic flow conditions. Alternatively, you may order your coverslips alone if you need to work in an open system (i.e. for AFM imaging).

Kit 1 contents (coverslips only):

> 5x standard (25 x 75 mm) chitosan-coated coverslips

Kit 2 contents (coverslips + sticky slides):

> 5x standard (25 x 75 mm) chitosan-coated coverslips

> 5x bottomless 6-channel sticky slides

All you need to have on your end is your culture medium of choice, deionized water and of course your favorite bacteria.

Depending on the bacterial species tested, a centrifugation step may help maximize bacterial adhesion on the Chitozen slide. You can also choose to add the centrifuging kit to your order if you want us to provide suitable centrifuge and racks that are compatible with sticky slides.

Centrifugation pack contents:

> µ-Slide Microscopy Rack (ibidi

> Magnetic Lid for Microscopy Rack

> Clamp & adapter for sticky slides

Check the table provided in the next questions for species-specific protocol recommendations.

Q/ Can I use Chitozen coverslips without the microfluidic chambers ?

Yes, the Chitozen coverslips can be used without the microfluidic chambers if your desired applications requires working in an open system (i.e. AFM microscopy), or if you would simply like more flexibility in your assay design. If using the Chitozen coverslips alone, separate wells can be created manually by using a sealing glue or Stencell silicon chambers. We can provide these 2 products for you. Contact us for more information.

Q/ Which bacteria have been successfully cultured on Chitozen?

The following species showed good adhesion and were successfully imaged on Chitozen coverslips: Escherichia coli, Caulobacter Crescentus, Corynebacterium glutamicum, Helicobacter pylori, Staphylococcus aureus, Vibrio cholerae, Myxococcus xanthus, Mycobacterium smegmatis, Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas fluorescens and Salmonella. You can find some example pictures taken on Chitozen slides on the product page.

Q/ How many experiments can I carry out with one Chitozen kit?

One kit contains what you need to prepare 5 Chitozen coverslips assembled with sticky-slides, each containing 6 independent channels. For each coverslip, you can either test up to 6 conditions at the same time or use one channel one day and the others later.

Q/ How long can I keep the Chitozen slides for?

Unmounted Chitozen coverslips can be stored up to 12 months at room temperature, in a dry place protected from direct sunlight. Exposure to high temperatures (>30°C) or brutal temperature variations can severely alter the chitosan coating and thus adherence efficiency.

Once mounted with a sticky-slide, Chitozen coverslips can be stored up to 2 months at room temperature, in a dry place protected from direct sunlight.

Q/ How long does it take to prepare a Chitozen slide?

Chitozen coverslip mounting with the sticky-slide is a very fast process that should take no more than 2 minutes. Once mounted, you will need around 30 minutes to prime your channels and load your samples before it is ready for imaging.

Q/ Is centrifugation necessary after loading the bacterial suspension?

Although centrifuging the slides usually maximizes bacterial adhesion to the Chitozen slides, a passive sedimentation protocol can be followed instead depending on the bacterial species tested. This is generally achieved by leaving the slide for 20 min on the benchtop after loading the bacterial suspension. Check out the Table below for a summary of current protocol recommendations for tested bacteria:

Bacterial species	Centrifugation	Passive sedimentation
Escherichia Coli	✅ (recommended)	✅
Bacillus subtilis		✅
Caulobacter Crescentus	✅	
Corynebacterium glutamicum		✅
Helicobacter pylori	✅	
Mycobacterium smegmatis	✅	
Myxococcus xanthus	✅	✅
Pseudomonas aeruginosa	✅	
Pseudomonas fluorescens		✅
Salmonella	✅	✅
Staphylococcus aureus		✅
Vibrio cholerae	✅	✅

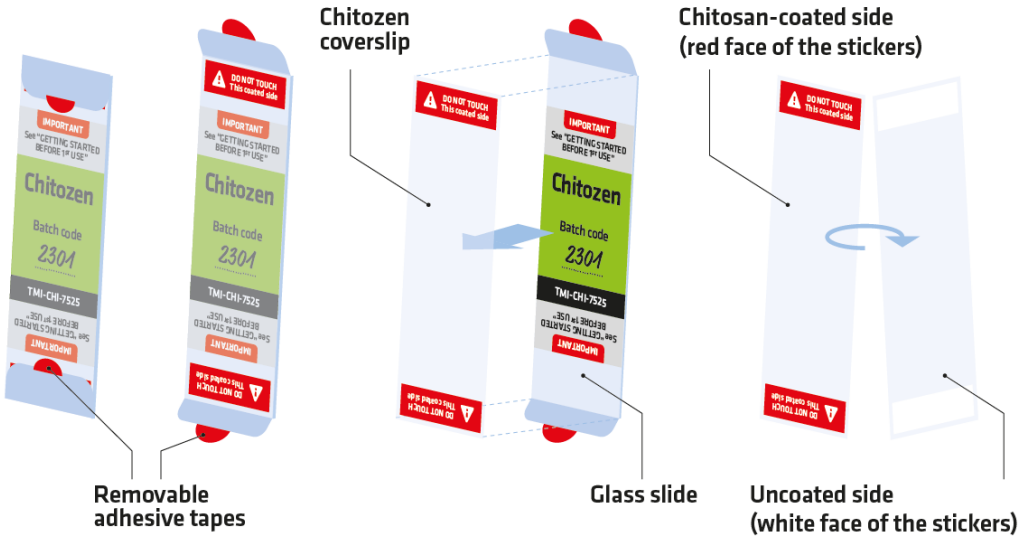
As a general rule, performing a centrifugation step will speed up bacteria sedimentation on the Chitozen slides, resulting in a flat cell monolayer. Passive sedimentation will be best suited for some assays that do not require monolayers (i.e. observing a flower-like pattern of collective migration from bacterial colonies).

Q/ How can I spot the side of the coverslip that is chitosan-coated?

Chitozen is composed of two different parts:

- The chitozen coverslip, with the chitosan-coated side exposed to the open air to avoid frictions that would damage the coating. Thus, it is the external side of the coverslip that is the one you must use, as indicated on the following scheme. Stickers will also help you find the coated side.

- A protective glass slide, on which a green label indicates the batch code.



Q/ What culture media should I use?

Adhesion to the Chitozen coverslips and bacterial motility on the chitosan surface are intrinsically linked to the culture medium used for loading them on the coverslips and throughout the experiment. Therefore, the choice of culture medium is critical for successful experiments (cf Table below). Careful validation will need to be performed if changing the medium composition, or working with a new bacterial species that has not been previously used with Chitozen.

Here is a recap table listing suitable culture media for each validated bacterial species:

Bacterial species	Culture medium
Escherichia coli	LB, M9, SOC
Salmonella	LB, M9, MgM
Pseudomonas fluorescens Vibrio cholerae	LB, M9
Mycobacterium smegmatis Bacillus subtilis Pseudomonas aeruginosa	LB, CYE
Myxococcus xanthus	CYE
Caulobacter crescentus	M2, PYE
Corynebacterium glutamicum Staphylococcus aureus	BHI
Helicobacter pylori	BB10

Q/ How can I maximize bacterial adhesion to the Chitozen coverslip?

A critical factor that can influence bacterial adhesion to the Chitozen slides is the choice of **culture medium** used to load the bacteria, and especially its **ionic strength**. You can find a list of bacterial species and culture media that have been successfully validated on Chitozen coverslips in the previous questions of this FAQ. If diluting the medium in water, make sure you use **fully deionized water** as increased ionic strength can alter bacterial adhesion.

Testing different **sedimentation techniques** (centrifugation or passive sedimentation) can also help maximize bacterial adhesion to the Chitozen coverslips. You can find a list of bacterial species and sedimentation techniques that have been successfully validated on Chitozen coverslips in the previous questions of this FAQ.

Q/ Which flow rate should I use to perfuse the system?

Assays with e.coli were successfully performed with flow rates ranging from 25 µL/min to 5 mL/min. We do not recommend applying a flow rate higher than 5 mL/min.

Q/ How can I minimize autofluorescence coming from the surrounding culture medium when imaging on Chitozen?

Using culture medium diluted ½ in deionized water, instead of full culture medium, can help reduce autofluorescence when imaging bacteria on Chitozen.

A Q&A updated in January 2024.