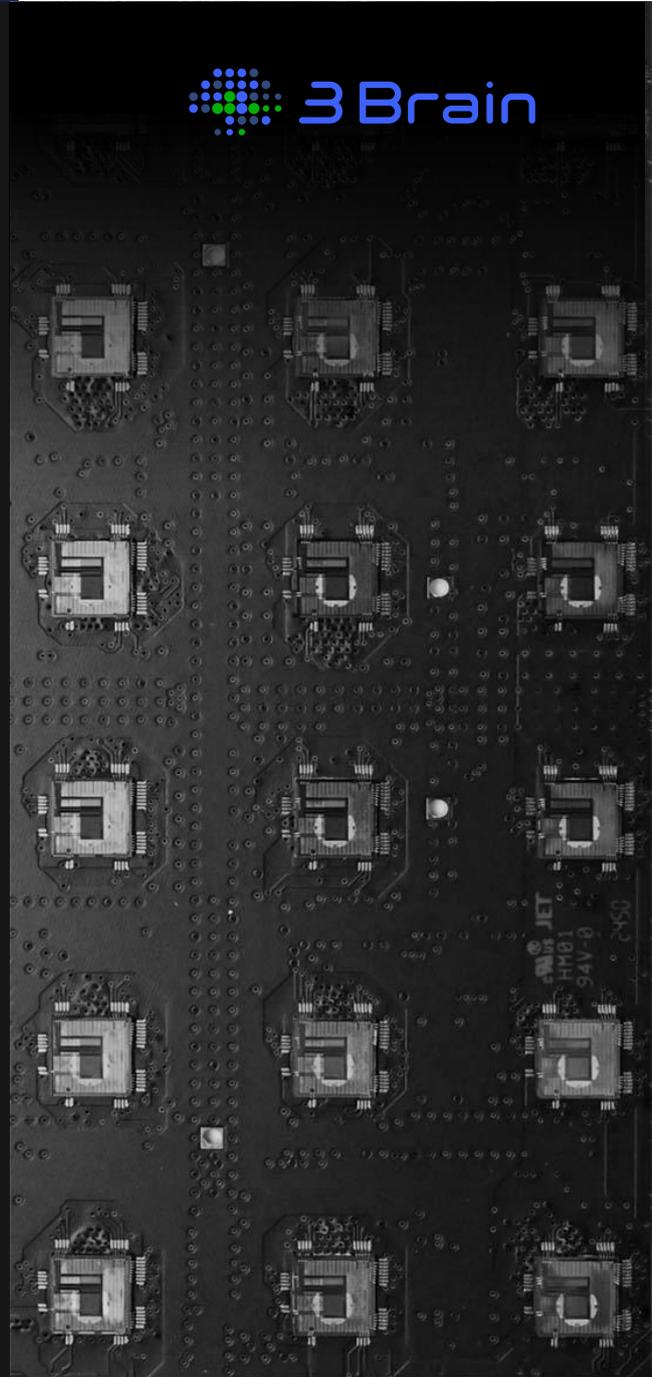


# Sample Holder 2 - User Guide for CorePlate™ 1W & 6W

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## Introduction

In order to maximise consistency whilst recording electrophysiological activity from 3D biological samples such as brain slices, organoids or spheroids, specialized sample holders are required to maintain maximal sample stability during recording to ensure robust and reproducible acute measurements. This document provides a comprehensive guide to the 3Brain sample holder set, highlighting its key benefits and offering step-by-step user instructions.

CorePlate™ 1W & 6W refer to the following:

- CorePlate™ 1W 38/60, 1W-3D 38/60/90, 1W 27/42, 1W 27/42L, 1W 51/81
- CorePlate™ 6W 38/60, 6W-3D 38/60/90

# The Sample Holder Set

Stable positioning is crucial when working with 3D biological samples such as brain slices, organoids, or neuronal spheroids. To address this challenge, we developed the **sample holder set** available in both single-well and six-well formats. It is fully compatible with the perfusion interface for both well formats and is designed to offer:

- **Improved signal.** The design optimizes coupling between the biological sample and electrodes, leading to enhanced signal quality and sensitivity.
- **Minimized displacement.** The holder prevents sample movement during long-term studies or compound testing, increasing results robustness.
- **Minimal damage.** The silicone net prioritizes biological sample integrity, prolonging sample viability during recording.

As shown in Figures 1 - 3, the sample holder set (for single-well and six-well systems) consists of four main components:

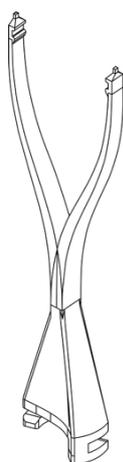
1. The Handling Tool.
2. The Silicone Net.
3. The Sample Holder Frame (hereby referred to as frame).
4. The Sample Holder Insert (hereby referred to as insert).

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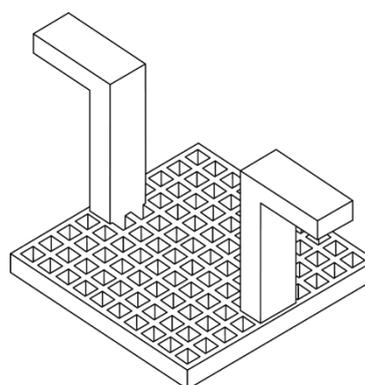
*The silicone net is compatible with all inserts.*

*All inserts are compatible with both frame formats.*

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Handling Tool



Silicone Net

Figure 1. Handling Tool and Silicone Net.

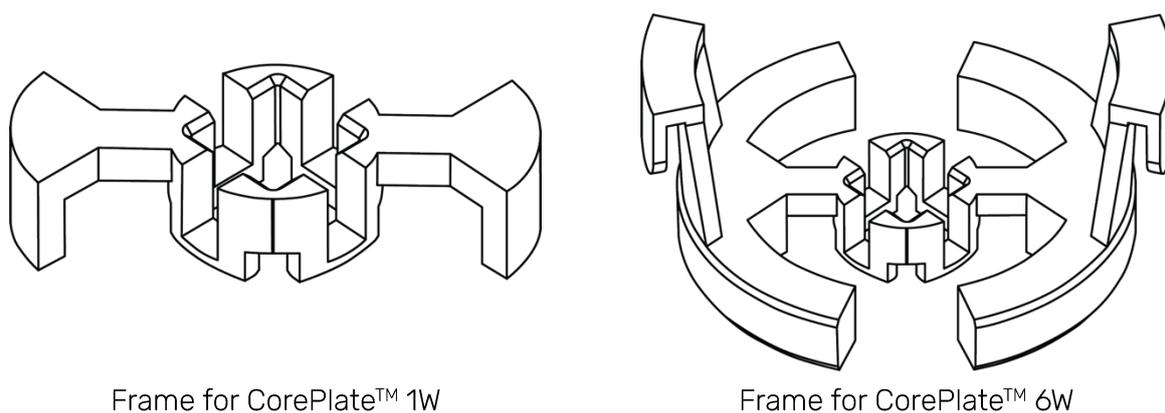


Figure 2. Frames for CorePlate™ 1 & 6W.

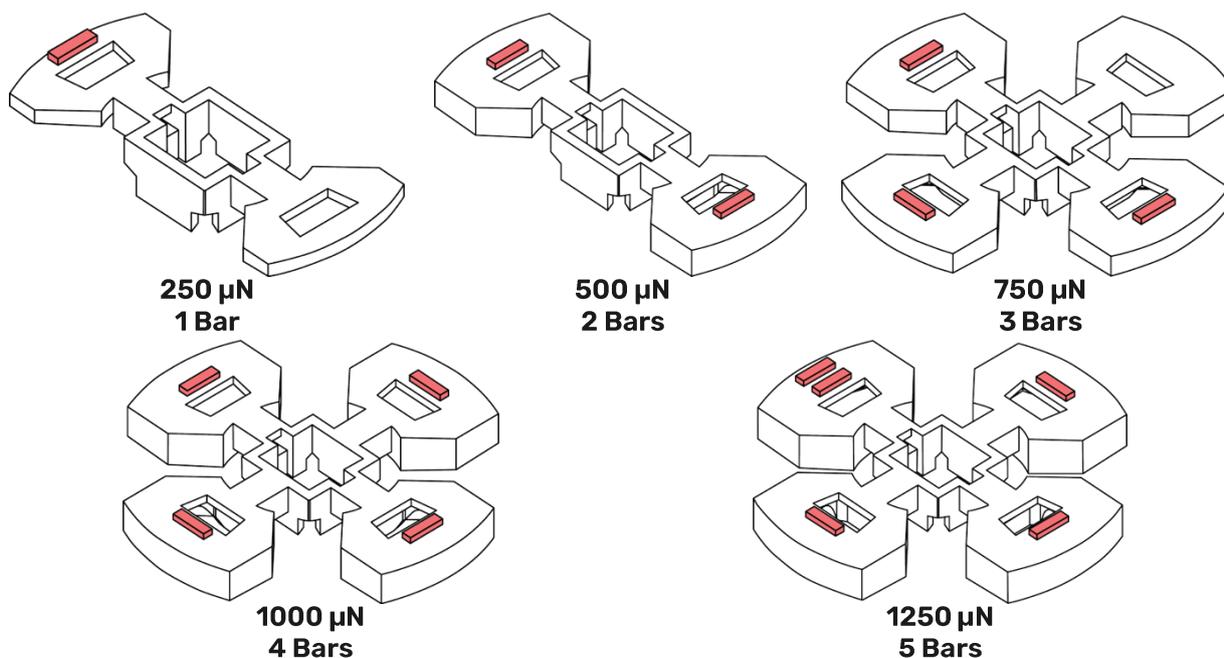


Figure 3. Inserts, the corresponding applied force and the number of bars (highlighted in red) on top of each insert.

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*The sample holder set is constructed with biocompatible materials. No signs of cytotoxicity were observed for extended periods (in-house evaluations indicate no cytotoxic response observed during a 30-day incubation period).*

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## Sample Holder Insert Selection

Sample holders inserts are offered in multiple force ratings, the visual differences and the number of bars on top of the insert can be seen in Fig. 3.

The most suitable force is dependent on the characteristics of the biological samples being analysed. Table 1 provides suggestions for the force rating for each biological model.

Table 1. Sample holder insert weights & corresponding biological model used

Biological Model	Sample Size* (µm)	Recommended Insert Force (µN)
<b>Spheroids</b>	<300	250
	300-700	1000
	700-1000	1250
<b>Organoids</b>	1000-1700	750
	>1700	500
<b>Brain Slices**</b>	220-300	1000

\* Refers to the diameter of brain spheroids and organoids, and the thickness of brain slices.

\*\* Refers to a typical thickness of 220 µm for cerebellar slices, and 300 µm for cortical-hippocampal and prefrontal cortex slices.

Note: These guidelines provide recommendations for optimal use. Effectiveness may vary depending on specific research parameters (e.g., age of the biological sample, thickness of the sample). Users should adapt these suggestions as needed for their unique research context.

## Cleaning and Sterilization of the Sample Holder Set

After each use, the sample holder set should be cleaned with ddH<sub>2</sub>O and/or 70% ethanol. If sterilization of the sample holder set is required, the following methods may be used.

- **Ethanol (Recommended)**  
Immerse all components of the sample holder set in 70% ethanol for 30 minutes. After ethanol sterilization, ensure to rinse the sample holder set abundantly with ddH<sub>2</sub>O.
- **UV Light**  
Only the sample holder frame, sample holder insert and handling tool may be sterilized with

UV light. Expose these components to UV-C light for 45 minutes, ensuring uniform exposure.

- **Autoclave**  
Only the silicone net is compatible with autoclaving.

## 1W Sample Holder Set Assembly and Use

After cleaning and sterilizing the sample holder set as required, assemble and use it with CorePlate™ 1W by following the steps below.

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*Placement of the biological sample will depend on its size. For smaller samples such as organoids and spheroids, we recommend positioning the biological sample on CorePlate™ 1W after the frame is in place. Large tissue samples (e.g. brain slices) may need to be positioned on CorePlate™ 1W before the frame is inserted. In both cases, the samples may remain fully immersed in media throughout the process of installing the frame and assembled insert / silicone net.*

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1. **Rinse the silicone net.** Rinse the net with a water-based solution (e.g. PBS, cell culture media etc.) to reduce hydrophobicity.
2. **Attach the silicone net.** Attach the silicone net in the centre of the sample holder insert utilizing the handling tool / tweezers or by hand (Fig. 4a & b). The handling tool possesses tips which help position the arms of the silicone net (highlighted in red in Fig. 4c).
3. **Soak the assembled silicone net / sample holder insert.** Leave the assembled silicone net and sample holder frame in a petri dish containing the solution from step 1.

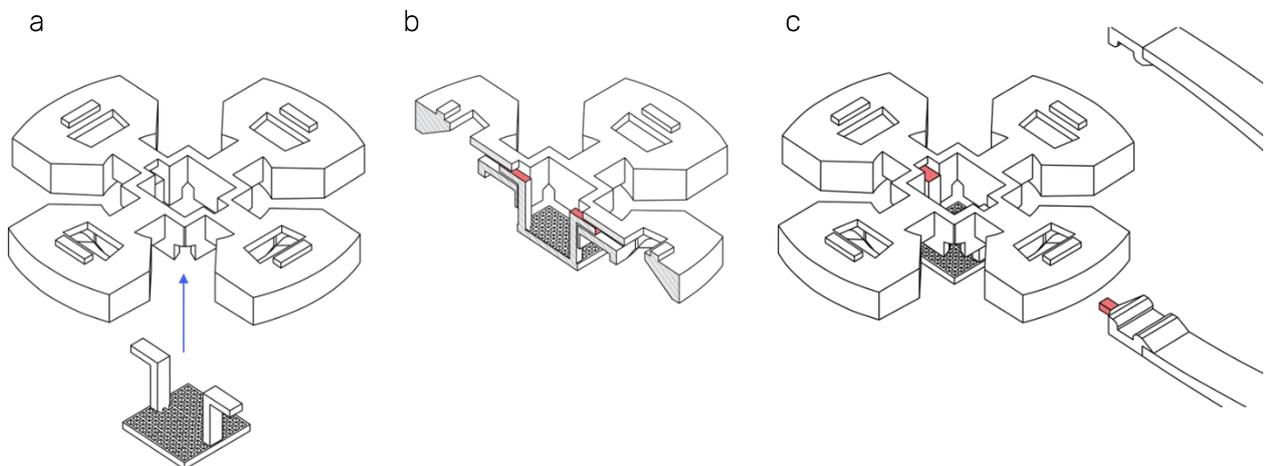


Figure 4. Positioning (a) and securing the silicone net into the insert (b). The handling tool features dedicated tips designed to align and seat the silicone-net arms, shown in red (c).

4. Insert the handling tool into the sample holder frame. Position the sample holder frame facing downwards. Insert the handling tool into the gaps of the sample holder frame (Fig. 5a)
5. Attach the sample holder frame to the handling tool. Rotate the handling tool counter-clockwise to secure it to the sample holder frame (Fig. 5b & c).

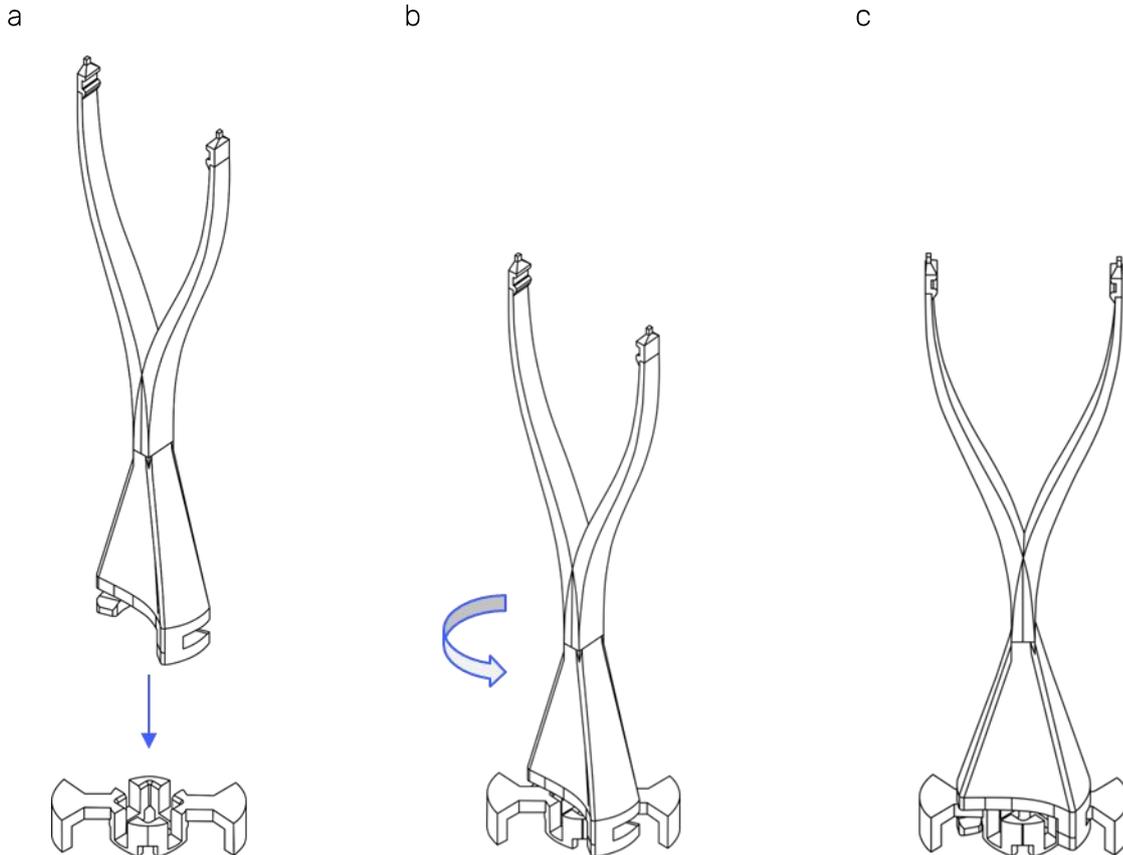


Figure 5. Inserting the handling tool into the frame (a), attaching the handling tool to the frame (b & c).

6. Insert the sample holder frame into CorePlate™ 1W. Gently push the assembled handling tool and sample holder frame into the well ensuring the indentations of the inside of the CorePlate™ 1W well (highlighted in red) align with the protrusions on the sample holder frame (Fig. 6a).
7. Remove the handling tool. Rotate the handling tool clockwise to detach the handling tool from the sample holder frame (Fig. 6b). When detached, remove the handling tool (Fig. 6c).

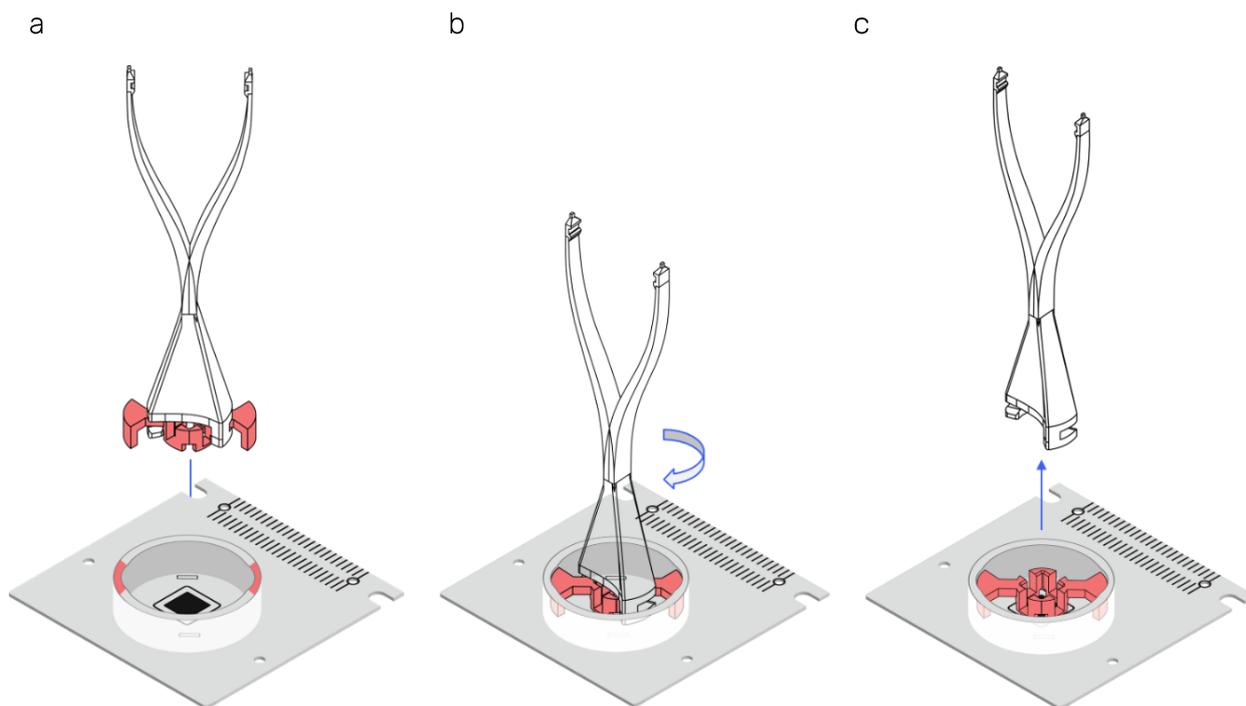


Figure 6. Inserting the Frame into CorePlate™ 1W (a), unlocking the handling tool (b) and removal (c) of the handling tool from the frame within CorePlate™ 1W.

8. Place the assembled sample holder insert / silicone net into the sample holder frame. Using the handling tool, lift the assembled insert / silicone net (Fig. 7a & b) from the soaking solution (step 3) and gently lower it into the frame (Fig 7. c).

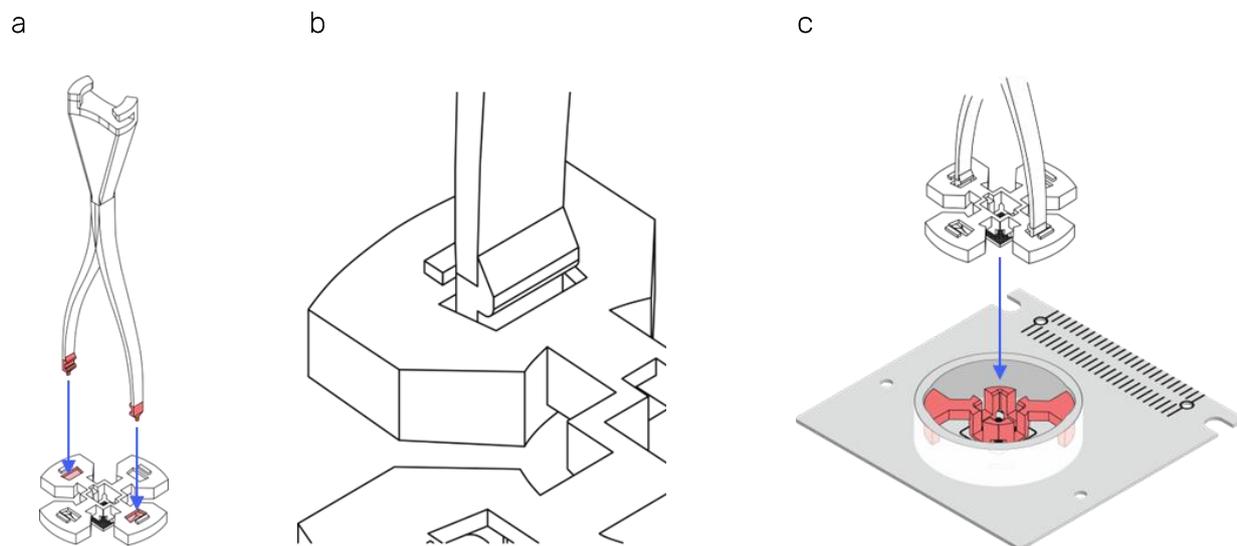


Figure 7. Using the handling tool to lift the insert (a, b) and place it into the frame (c).

## 6W Sample Holder Set Assembly and Use

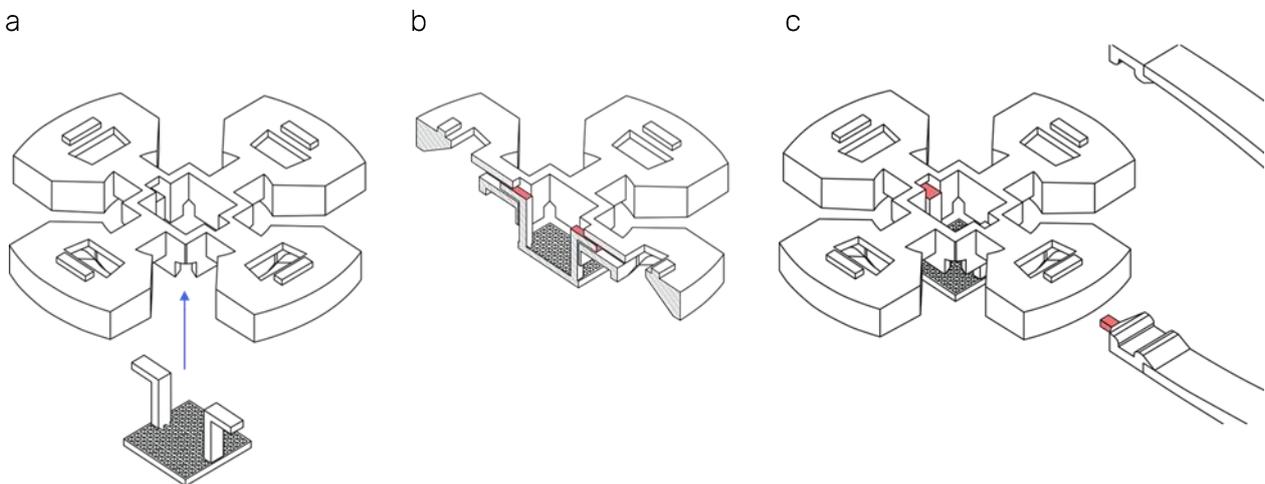
After cleaning and sterilizing the sample holder set as required, assemble and use it with CorePlate™ 6W by following the steps below.

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*Placement of the biological sample will depend on its size. For smaller samples such as organoids and spheroids, we recommend positioning the biological sample on CorePlate™ 6W after the frame is in place. Large tissue samples (e.g. brain slices) may need to be positioned on CorePlate™ 6W before the frame is inserted. In both cases, the samples may remain fully immersed in media throughout the process of installing the frame and assembled insert / silicone net.*

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1. **Rinse the silicone net.** Rinse the net with a water-based solution (e.g. PBS, cell culture media etc.) to reduce hydrophobicity.
2. **Attach the silicone net.** Attach the silicone net in the centre of the sample holder insert utilizing the handling tool / tweezers or by hand (Fig. 8a & b). The handling tool possesses tips which help position the arms of the silicone net (highlighted in red in Fig. 8c).
3. **Soak the assembled silicone net / sample holder insert.** Leave the assembled silicone net and sample holder frame in a petri dish containing the solution from step 1.



*Figure 8. Positioning (a) and securing the silicone net into the insert (b). The handling tool features dedicated tips designed to align and seat the silicone-net arms, shown in red (c).*

4. **Insert the handling tool into the sample holder frame.** Position the sample holder frame facing downwards. Insert the handling tool into the gaps of the sample holder frame (Fig. 9a).
5. **Attach the sample holder frame to the handling tool.** Rotate the handling tool counter-clockwise to secure it to the sample holder frame (Fig. 9b & c).

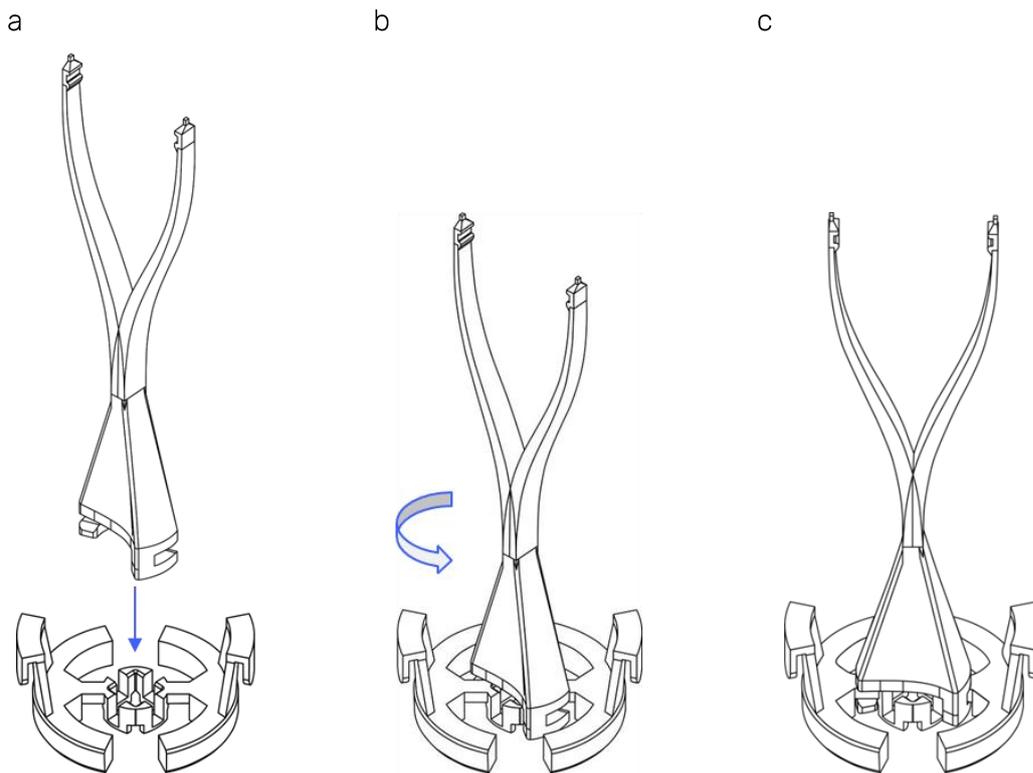


Figure 9. Inserting the handling tool into the frame (a), attaching the handling tool to the frame (b & c).

6. Insert the sample holder frame into CorePlate™ 6W. Gently push the assembled handling tool and sample holder frame into the well ensuring the indentations of the outside of the CorePlate™ 6W well (highlighted in red) align with the protrusions on the sample holder frame (Fig. 10a).
7. Remove the handling tool. Rotate the handling tool clockwise to detach the handling tool from the sample holder frame (Fig. 10b). When detached, remove the handling tool (Fig. 10c).

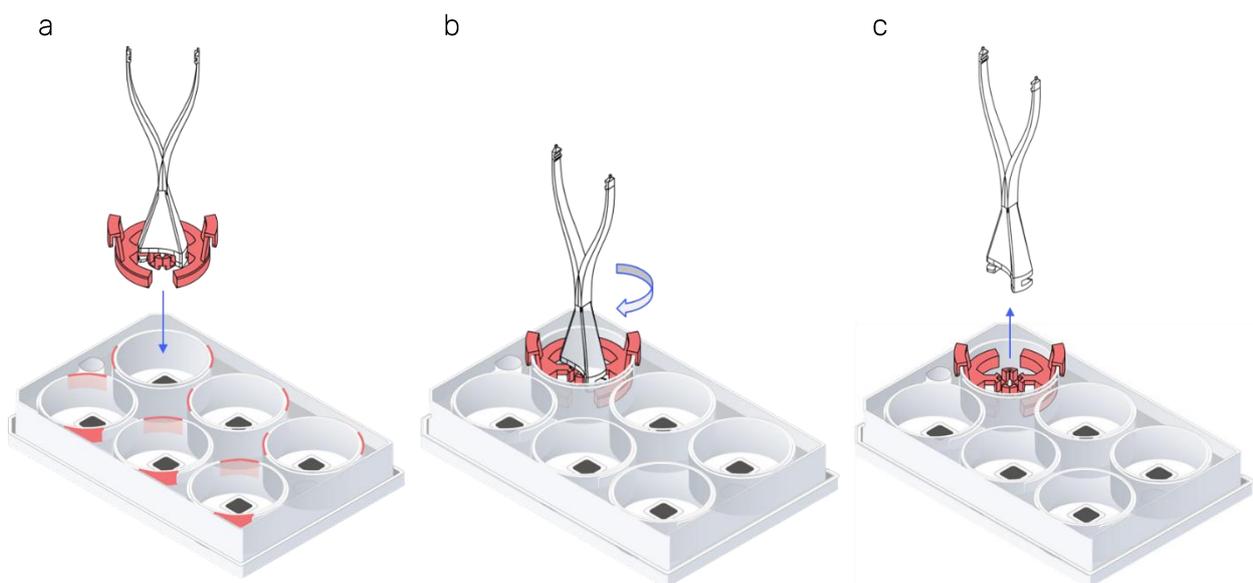


Figure 10. Inserting the Frame into CorePlate™ 6W (a), unlocking the handling tool (b) and removal (c) of the handling tool from the frame within CorePlate™ 6W.

- Place the assembled sample holder insert / silicone net into the sample holder frame. Using the handling tool, lift the assembled insert / silicone net (Fig. 11a, b) from the soaking solution (step 3) and gently lower it into the frame (Fig. 11c).

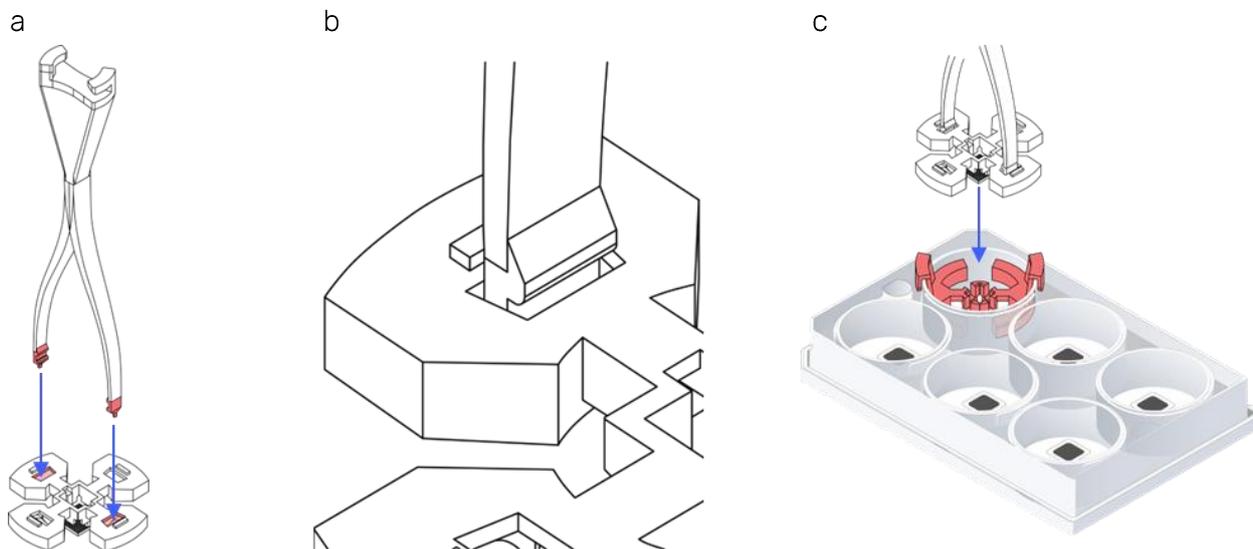


Figure 11. Using the handling tool to lift the insert (a, b) and place it into the frame (c).

### Disclaimer

These guidelines are intended to serve as suggestions and recommendations rather than strict rules. The effectiveness of the tools mentioned may vary depending on specific circumstances and experimental conditions (i.e., age of the biological model, thickness of the slice). Users are encouraged to consider these guidelines as informative resources, exercising their judgment and expertise in adapting them to their unique research contexts.

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*Note: These guidelines are subject to continuous improvement and updates based on evolving scientific knowledge and technological advancements.*

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## Support

For any further questions, please contact our Customer Success team at [cs@3brain.com](mailto:cs@3brain.com)