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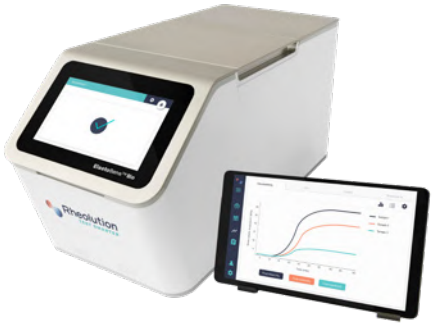
**Technical note** | ElastoSens™ Bio



ELASTOSENS™ BIO

TIME MEASUREMENTS

THERMO STIMULATION

TESTING CONFIGURATION

HYDROGELS

# How to test thermosensitive hydrogels using µ-volume sample holder of the ElastoSens™ Bio?

## EXPANDING POSSIBILITIES FOR VISCOELASTIC TESTING OF LIMITED VOLUMES OF HYDROGELS WITH THE µ-VOLUME SAMPLE HOLDER

Hydrogels play a pivotal role in the biomedical field due to their versatile applications, ranging from drug delivery systems to tissue engineering. The mechanical characterization of hydrogels is crucial to assess its fit with the final application and for understanding their performance over time. However, obtaining a sufficient quantity of samples for accurately testing them can be a challenge. In response to this limitation, the µ-volume sample holder of the ElastoSens™ Bio was developed to test these types of limited materials such as natural polymers, blood, plasma, and cellularized hydrogels.

The µ-volume sample holder is designed to be entirely autoclavable and to fit into a 12-well plate, facilitating convenient sample storage. This characteristic, coupled with the non-destructive nature of ElastoSens™ Bio technology, enables the repeated testing of the same sample. This capability allows for the tracking of its mechanical behavior over time within controlled environments.

One of the first steps for testing thermosensitive hydrogels involves the preparation of their liquid form, followed by introducing them into the holder and allowing gelation under the specified environmental conditions. These steps play a crucial role in ensuring the accuracy and quality of the data collected by the instrument. In this light, a comprehensive protocol for preparing and testing thermosensitive hydrogels using the µ-volume sample holder of the ElastoSens™ Bio will be described in this technical note, followed by examples of results obtained with gelatin, agar and pluronic F127.

## PREPARING AND TESTING THERMOSENSITIVE HYDROGELS IN THE µ-VOLUME SAMPLE HOLDER OF THE ELASTOSENS™ BIO

Hydrogels can be inserted into the µ-volume sample holder using the methodology 1 outlined in the tutorial video and in a previous technical note (as well as in its user guide). The following section provides a comprehensive set of cautionary points and recommendations, guiding the process from loading to testing thermosensitive hydrogels in the ElastoSens™ Bio:

1) For certain versions of the µ-volume sample holder device, users need to add the ring when not using the plastic spring base (**Figure 1**). Opting for the ring might be preferable for a number of thermosensitive hydrogels, especially considering potential challenges in removing the plastic base later on (refer to the user guide and the previous technical note for additional recommendations on its removal).

2) It is extremely important to ensure thorough penetration of the hydrogel into the inner cavity of the holder (**Figure 2**). Achieving proper loading involves a slow pipetting technique, allowing the user to visually confirm the hydrogel's entrance into the cavity. Pre-incubating the loading system at the same temperature of the liquid sample can further prevent rapid solidification at specific points, promoting a more homogeneous distribution.

3) Once properly loaded into the µ-volume sample holder, the sample needs to be immersed in liquid media as soon as possible to avoid drying (at the desired gelation temperature). Even if not readily perceptible by eyes, a 250-uL hydrogel sample dries considerably as soon as it is in contact with air. It is recommended that the sample is immersed in liquid media right after the transition point (as soon as the gel is able to support itself over the spring). It is the user's responsibility to find the necessary time to reach the transition point before adding the sample into the liquid media.

4) Before the first test for a given sample, either the entire loading system or solely the µ-volume sample holder with the sample can be introduced into liquid media (**Figure 3**). It is advisable for the user to experiment and determine the optimal approach for the given sample. Whether using the loading system or the µ-volume sample holder with the sample, it is essential to place it on a flat surface to ensure uniform solidification.

5) The sample needs to be in the liquid media at the desired temperature long enough to reach its final shear storage modulus (G') before its first test in the ElastoSens™ Bio. The duration required to reach the final stiffness (G') depends on the material. The user can measure the gelation kinetics with the macro holder before to have an idea about this duration. It is the user's responsibility to find the necessary time to reach the final G' before testing the sample in the ElastoSens™ Bio.

**Figure 1: Loading system with plastic spring base (left) and with ring (right). The ring is preferred over the plastic spring base for a number of thermosensitive hydrogels.**

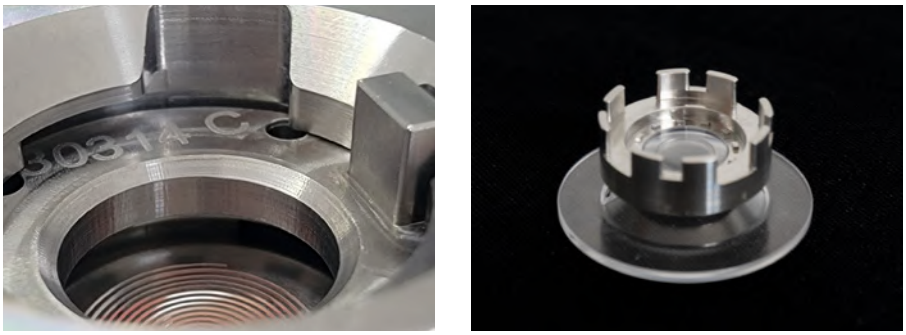


**Figure 2: Inner cavity of the µ-volume sample holder seen from the top when using methodology 1 (left), and seen from the bottom for illustration (right).**

**Figure 3: Entire loading system (left) and the µ-volume sample holder (right) immersed in liquid media.**

6) The µ-volume sample holder containing the sample needs to be free of liquid media when testing in the ElastoSens™ Bio. The user can gently use absorbent paper or sterile gauzes to remove the excess of liquid media that accumulates on the top and bottom of the µ-volume sample holder. It is important to not touch the formed hydrogel to avoid damaging it.

7) When testing a thermosensitive hydrogel in the µ-volume sample holder (once the final G' is reached), it is recommended to apply no more than 2 minutes of test duration to avoid sample drying which can falsely increase the G' (**Figure 4**).

8) After testing, the µ-volume sample holder containing the sample should be immediately transferred back to the liquid media if the user wants to test it again at later time points, using for example a 12-well plate. Ensure that the bottom of the sample (below the spring) is properly covered by the liquid media.

9) Clean the components of the µ-volume sample holder thoroughly with soap and running water. Allow the holder to dry completely before storing them. It is advisable to follow these recommendations to ensure the longevity and integrity of the stainless steel components.

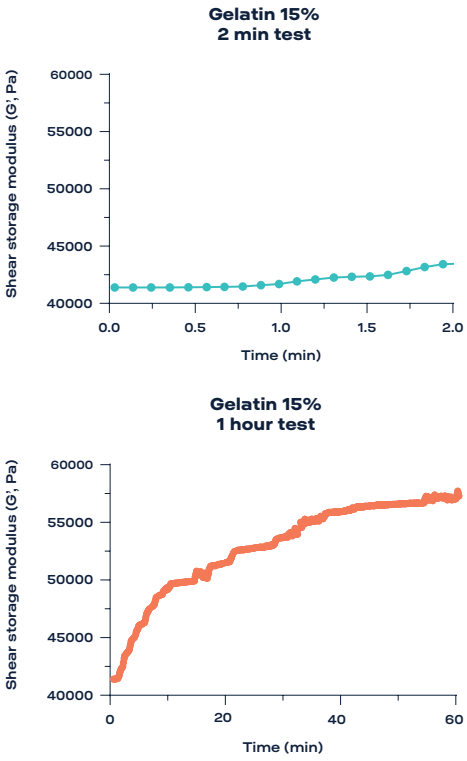



**Figure 4: Gelatin 15% incubated in PBS at 20 °C for 1.5 h and tested for 2 and 60 min in the ElastoSens™ Bio after incubation. The increase in G' over time demonstrates the drying of the sample.**

## SHEAR STORAGE MODULUS (G') OF GELATIN, AGAR AND POLOXAMER

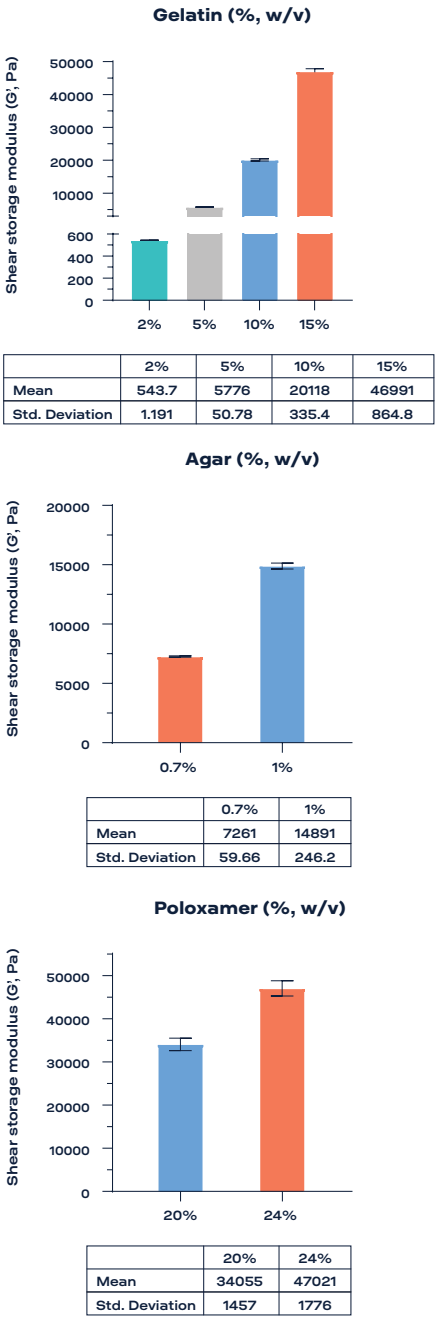
Gelatin type B from bovine skin, Agar and Pluronic F-127 (Sigma-Aldrich, USA) were prepared at different concentrations in distilled water and tested in the ElastoSens™ Bio following the guidelines of this technical note. Briefly, the liquid solutions were poured into the µ-volume sample holder using the methodology 1 without the plastic spring base. The loading systems were always pre-incubated at the temperature of the liquid solution. Additional detail of their protocol can be found in the table below with an explanatory section before. All samples were tested in the ElastoSens™ Bio for 2 minutes with a temporal step of 5 s.

1) Liquid sample temperature: refers to the temperature at which the gelatin, agar, and polymer was maintained until their addition into the µ-volume sample holder
2) Gelation temperature and duration: refers to the temperature at which the entire loading system with the sample was exposed right after sample loading and the total duration before adding it into liquid media (next step below).
3) Incubation time and temperature: refers to the temperature and duration at which the sample was incubated in liquid media (gelatin and agar were added in PBS). This is the step where the sample will achieve its final G' prior to testing it in the ElastoSens™ Bio.
4) Total time until testing: approximately total time from sample loading into the µ-volume sample holder until the starting of the test in the ElastoSens™ Bio. It is basically the duration of step 2 plus the duration of step 3.

Average results are expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism (GraphPad Prism Software, USA) using unpaired t test with Welch's correction. Significance was retained when $p < 0.05$.

**Table 1: Additional detail of the protocol used for testing gelatin, agar and pluronic F-127.**

| Sample type, concentration (%, w/v) | Liquid sample temperature | Gelation temperature and duration | Incubation temperature and duration | Total time until testing |
|---|---|---|---|---|
| Gelatin, 2 (n=4) | 40 °C | 4 °C, 20min | 4 °C, 2h30 | 2h50 |
| Gelatin, 5 (n=3) | 40 °C | 4 °C, 15min | 4 °C, 2h | 2h15 |
| Gelatin, 10 (n=6) | 40 °C | 4 °C, 10min | 4 °C, 1h30 | 1h40 |
| Gelatin, 15 (n=5) | 40 °C | 4 °C, 5min | 4 °C, 1h | 1h05 |
| Agar, 0.7 (n=4) | 40 °C | 4 °C, 20min | 4 °C, 1h | 1h20 |
| Agar, 1 (n=4) | 40 °C | 4 °C, 20min | 4 °C, 40min | 1h |
| Pluronic, 20 (n=3) | 4 °C | 40 °C, 30min (this material is easily dissolved in liquid media, so anti-evaporation oil was added to the sample to reduce sample drying) | | 30min |
| Pluronic, 24 (n=3) | 4 °C | 40 °C, 30min (this material is easily dissolved in liquid media, so anti-evaporation oil was added to the sample to reduce sample drying) | | 30min |




| | 2% | 5% | 10% | 15% |
|---|---|---|---|---|
| Mean | 543.7 | 5776 | 20118 | 46991 |
| Std. Deviation | 1.191 | 50.78 | 335.4 | 864.8 |




| | 0.7% | 1% |
|---|---|---|
| Mean | 7261 | 14891 |
| Std. Deviation | 59.66 | 246.2 |




| | 20% | 24% |
|---|---|---|
| Mean | 34055 | 47021 |
| Std. Deviation | 1457 | 1776 |

**Figure 5: Average shear storage modulus (Pa) obtained for gelatin, agar and poloxamer using the µ-volume sample holder.**

**Figure 5** displays the average shear storage modulus (measured in Pa) for gelatin, agar, and pluronic at the different concentrations tested. The small standard deviation (always lower than 4 %) demonstrates the good repeatability of the measurements. Interestingly, Pluronic F127 exhibited the highest standard deviation values, possibly attributed to increased susceptibility to drying from the bottom surface (due to the anti-evaporation oil being added only to the top of the sample as shown in the table 1). Statistical differences were consistently observed between different concentrations of the same material ($p < 0.001$).

Overall, the results of this study show the robustness of the protocol for testing thermosensitive hydrogels.

## KEY TAKEAWAYS

— This technical note provides recommendations for testing thermosensitive hydrogels in the ElastoSens™ Bio using the µ-volume sample holder with high accuracy and repeatability.

— Hydrogels can quickly dry upon exposure to air, and therefore, managing this phenomenon is essential for the accurate and repeatable results.

— Given the variability of thermosensitive hydrogels, it is advisable to practice and adjust the protocol for sample preparation, loading into the holder, and testing in the instrument, following the recommendations of this technical note and the referred supporting materials.