

Matrigen Softwell®

Frequently Asked Questions



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Matrigen Softwell[®] Hydrogels

General Questions

- **What is Softwell[®]?**

Softwell[®] is a transparent hydrogel bound to the surface of any cell culture well. It is much softer than tissue culture plastic. Softwell[®] replicates a broad range of tissue stiffness, so you can culture neurons in an environment as soft as the brain, cardiomyocytes on the stiffness of muscle, or whatever your cells' tissue of origin may be. Importantly, this platform can be adapted to most cell-based assays and detection methods, so you can make cell culture more realistic without making it more complicated.

- **How can I order?**

Matrigen products can be ordered in Europe on Cell Guidance Systems website www.cellgs.com or by sending an e-mail to order@cellgs.com. All the products are made to order, which means delivery will typically take 2 – 3 weeks. In other regions, please contact info@matrigen.com.

- **Do you provide smaller pack sizes for initial testing?**

Matrigen products are sold as single plates or packs of 10 dishes/plates.

- **What is the composition of the hydrogels?**

Softwell[®] hydrogels are made of polyacrylamide crosslinked with bisacrylamide. Polyacrylamide is a non-toxic polymer which forms a soft gel when hydrated. The physical properties of these hydrogels can be modified allowing to control the stiffness. Moreover, polyacrylamide hydrogels may be chemically conjugated with ECM ligands to enable cell attachment, growth and differentiation.

- **Are the hydrogels manufactured under sterile or aseptic conditions?**

All products are manufactured and packaged under aseptic conditions. Plates are individually wrapped in foil pouches.

- **What is the storage and shelf life of the products?**

All products are individually packed and labelled with a manufacturing date. As long as the hydrogels remain sealed or hydrated, all products are stable for 6 months from the date of receipt. Non-Activated and Easy Coat™ recommended storage temperature is 4 - 25°C. Collagen coated hydrogels should be stored at 4°C. Hydration is critical, so keep the hydrogels in the sealed package until ready for use. Adhesion Free products may be stored at room temperature and do not require hydration.

- **Is it possible to open the package and use different wells at different times?**

Yes, this should be possible. To make sure that the hydrogels are kept hydrated, the wells should be filled with PBS until you are ready to use them.

- **What is the range of available stiffness values?**

Softwell® hydrogels are available in 9 standard stiffness values (0.1, 0.2, 0.5, 1, 2, 4, 8, 12, 25, 50 and 100 kPa and a high-throughput screen option (range of standard stiffness values).

- **What are the stiffness values for plastic and glass?**

The stiffness values for polystyrene and glass are about 3,000,000 kPa and 60,000,000 kPa, respectively, which makes these materials much stiffer than the hydrogels.

- **Is it possible to have hydrogels with other stiffness values?**

Different stiffness values can be manufactured upon request. An extra charge for custom products will be applied.

- **What format are Softwell® hydrogels available in?**

Softwell® hydrogels are available in multi-well polystyrene plates, multi-well glass bottom plates, polystyrene dishes, glass bottom dishes, and standard plastic plates with removable coverslips. For more information, please visit the [Matrigen Product Selection Guide](#).

- **Which coatings are available in Softwell® hydrogels?**

Softwell® hydrogels are available as Non-Activated, Easy Coat™, Collagen and Adhesion Free™. Non-Activated hydrogels can be used as low protein and cell attachment surface or alternatively as a substrate that can be chemically activated by your own methods. Easy Coat™ hydrogels are activated to bind to any extracellular matrix proteins. Collagen hydrogels are a ready to culture option, since the hydrogels are bound to type I collagen. Adhesion Free™ is a special surface treatment option in which hydrogels resist protein adsorption and are 100% non-adherent to cells.

- **What is the chemistry used on Easy Coat™ hydrogels?**

The exact chemical treatment used on Easy Coat™ hydrogels is proprietary. But essentially, the treatment populates the hydrogel (or surface) with quinone groups, which form covalent bonds with primary amines, thiols, or strong nucleophiles. This means that the activated hydrogels will bind to any peptide or protein. The hydrogels are ready to coat with the ECM protein of choice, e.g. collagen (types I, III and IV), fibronectin, laminin, and poly-lysine. Be aware that once all the binding sites are reacted, additional molecules will not bind directly to the hydrogel.

- **What is the Collagen used for Collagen coated-dishes?**

All collagen-coated products use rat tail collagen type I. Bovine skin type I collagen can be used upon request. All hydrogels are incubated with a solution of 0.02 mg/ml with a constant surface density of collagen across different stiffness values. The exact concentration of collagen in all hydrogels has not been measured.

- **Do you have any experience using hydrogels with other ECM proteins besides Collagen I?**

Based on customers' experience, Softwell® hydrogels bind well to different ECM proteins, including fibronectin, laminin, collagen IV, among others. The hydrogels bind quite readily to the first protein that they are exposed to. Please note that binding normally occurs at a pH range from 6 to 9. We recommend the use of PBS for resuspending the ECM protein of interest.

- **What are the exact dimensions of the plates provided?**

For setting up some experiments, it is important to understand the exact dimensions of the plates provided by Matrigen. If this information is required, please send an e-mail to info@cellgs.com. The standard glass thickness used is #1.5, but #0 can be used upon request.

- **Is it possible to transfer single coverslips to other multi-well plates without damaging the hydrogel?**

The coverslips are smaller than the respective well size and can be carefully removed, while immersed, by using fine-tipped tweezers.

- **What is a High-Throughput Screen (HTS) plate?**

The HTS plates are designed to screen the standard stiffness values. Each row will have the following stiffness values: 0.1, 0.2, 0.5, 1, 2, 4, 8, 12, 25, 50 and 100 kPa, and one well without hydrogel. The well without hydrogel will have the same treatment as the hydrogels, which can either be Easy Coat™ or Collagen.

- **Is Softwell® like Matrigel™?**

Hydrogels <1kPa are as soft as Matrigel™. But whereas Matrigel™ is comprised of tumor-derived proteins and growth factors, Softwell® is based on a synthetic polymer.

- **Will the hydrogels detach from the well?**

Hydrogels should remain bound under most conditions, including long-term cell culture. Extra care should be taken during pipetting and aspiration steps for hydrogels <2 kPa.

Cell culture and experimental questions

- **Are the hydrogels safe for my cells?**

Matrigen products are certified non-cytotoxic and pyrogen free.

- **Are the hydrogels compatible with my current assays?**

Softwell® hydrogels are compatible with conventional biological assays and detection methods. The hydrogels are thin allowing easy washing away of reagents and antibodies. Moreover, the hydrogels are optically transparent, meaning that the hydrogels will not interfere with phase contrast or fluorescence microscopy.

- **How do I remove my cells from the hydrogels?**

Cells can be detached and passaged with standard reagents and protocols, just as on tissue culture plastic. The gel is bound to the substrate and will resist detachment.

- **There is condensation on my plate. Is this normal?**

Since the gels are composed of 95–99% water, there will almost always be some condensation on the lid and gel surface. The presence of condensation is actually a good sign that the gels have remained hydrated, with the foil pouch acting as an effective moisture barrier.

- **How do I keep gels hydrated after opening the foil pack?**

The gels should never be allowed to dry, especially with the lid removed for an extended period of time, as this can dry the gel and introduce contamination even in a laminar flow hood. Instead, the gels should be immersed in liquid immediately after opening the foil pouch and opening times minimized.

- **Can I detach my cells with trypsin?**

Yes, but Matrigen recommends incubating the hydrogel in serum-free media or buffer for ≥30 minutes at 37°C to remove serum absorbed in the gel prior to adding trypsin. To bypass this step, use TryPLE™ (ThermoFisher Scientific), which dissociates cells in serum-containing conditions.

- **Following trypsinization, I still cannot extract my cells from the hydrogel. What should I do?**

Some cell types are difficult to extract as they attach strongly to the hydrogel matrix. If the use of TryPLE™ is not sufficient, we recommend the use of the stronger TryPLE™ Select 10X (ThermoFisher Scientific).

- **Can cells be scraped from the hydrogels?**

A cell scraper with a silicone rubber blade will do the trick (available from CytoOne®). Scrape with gentle, short strokes to avoid disrupting the hydrogel.

- **Can I fix my cells?**

Softwell® is compatible with fixatives such as formalin, glutaraldehyde, and methanol. Note that dehydrating agents will cause the hydrogel to turn opaque, but regains transparency upon hydration.

- **Is there a recommended protocol for the coating of Easy Coat™ hydrogels with ECM proteins?**

We recommend the use of PBS for resuspending the ECM protein of interest. For coating the hydrogels, we suggest diluting collagen I in PBS at a concentration of 10 µg/ml. For other proteins, the recommended concentration will be much lower (e.g. fibronectin and laminin would be 100-1000 ng/ml). Volume per well would be enough to cover the gel. Finally, a 30 minute incubation at room temperature or longer is recommended.

- **Will collagen solubilised in acetic acid be detrimental to the hydrogel?**

Acidic solutions will not affect Softwell® hydrogels.

- **What is the recommended product for immunofluorescence staining?**

For immunofluorescence staining, the Softslip™ (hydrogels bound to glass coverslips in multi-well plate) products are ideal since the coverslips can be removed from the well and inverted on a glass slide. Hydrogels bound to glass bottom dishes and 96-well glass bottom plates are also suitable, but keep in mind that the thickness of the hydrogel (60 to 500 µm) will limit the resolution for 40x and 63x objectives. Hydrogels bound to polystyrene multi-well plates and dishes are also compatible for immunofluorescence, but the thickness of the plastic and gel will also limit the resolution for 40x and 63x objectives.

- **Can I image my cells?**

As mentioned above, the hydrogels are transparent and compatible with brightfield, phase contrast, and fluorescence microscopy. The combined thickness of the hydrogel and substrate is within the focal range of 40X objectives. For higher magnifications, use Softslip™ hydrogels, which can be inverted onto a coverslip.

- **Should I expect auto-fluorescence from Softwell® hydrogels or from antibodies binding to the hydrogels when performing immunofluorescence assays?**

Softwell® hydrogels do not have any associated auto-fluorescence and they will not bind to anything besides the ECM proteins used for cell adherence. To ensure the removal of unbound detection

molecules, perform wash steps 3x longer than directed by standard protocols. It is also recommended to use 1% BSA and/or 5% goat serum as a blocking buffer to minimize any background in the images.

- **Do Softwell® hydrogels keep a consistent focal plane?**

Softwell® hydrogels have a uniform surface, so a constant focal plane can be maintained if the plate is represented by a single stiffness. However, more adjustments might be required with variable stiffness plates, because the hydrogels swell to a different extent according with their stiffness (softer gels swell more than stiffer gels).

- **How do I isolate RNA?**

Matrigen recommends using a spin-based kit such as RNeasy® (Qiagen). Incubate the hydrogel with lysis buffer for 10 minutes on ice, collect the lysate, and repeat to recover RNA absorbed in the gel.

Quality control technical questions

- **What is the methodology for stiffness calibration per batch?**

Stiffness calibration and Young's Modulus calculus are performed by applying Hertz's sphere model (assuming a Poisson ratio of 0.48 for polyacrylamide) after the measurement of indentation of a < 1 mm carbide steel sphere (density = 15.63 g/cm³) upon the surface of the hydrogel. The measurements are taken while the hydrogel and sphere are immersed in phosphate-buffered (PBS) (density = 1 g/cm³). This procedure is applied to each batch, meaning that each time a stock (pre-polymerization) solution of a targeted stiffness is made, an aliquot is polymerized for stiffness validation by using the above described method.

- **Is stiffness calibration performed with Atomic Force Microscopy (AFM)?**

Stiffness calibration is performed by applying Hertz's theory as described in the previous question. The obtained values are well correlated with AFM measurements. AFM thickness validation is not routinely performed.

- **What is the calibration for gel thickness?**

The gel thickness may vary depending on stiffness and format within a range of 200 and 500 µm. For hydrogels on polystyrene, the thickness is ~ 500 µm, whereas for hydrogels on Softslip™, 6, 12, 24, and 96 well glass bottom plates, and on Softview™, the thickness is ~100 µm. The hydrogels are no thicker than 500 µm to minimize the interference of the gel in microscopy and other assays. The gel thickness can be modified upon request. The stiffness of the hydrogel does not change after coating.

- **What is the size of the pores in the hydrogels?**

All the pores in the hydrogels are $<0.2 \mu\text{m}$. To ensure that this parameter is satisfied, the hydrogels are overlaid with $0.2 \mu\text{m}$ fluorescent beads to confirm that they cannot penetrate the softest hydrogels.

- **Do you perform electron microscopy to analyze hydrogel morphology?**

Matrigen has not performed electron microscopy analysis on the hydrogels.

- **What is the methodology for quality and uniformity control of the collagen coated hydrogels?**

The surface density of collagen is relatively constant across stiffness. This uniformity was confirmed by using chemiluminescence-based detection of gel-bound collagen across a range of different stiffness values.

SoftTrac™ Products

- **What are SoftTrac hydrogels?**

SoftTrac™ are hydrogels with fluorescent microspheres immobilized at the surface.

- **What will be the use of SoftTrac hydrogels?**

SoftTrac™ hydrogels main use is for traction force microscopy.

- **What microspheres are immobilized in the hydrogels?**

Matrigen provides hydrogels with the following range of microspheres: $0.2 \mu\text{m}$ yellow/green, $1 \mu\text{m}$ yellow/green, and $1 \mu\text{m}$ red microspheres. Yellow-green fluorescent microspheres (0.2 or $1 \mu\text{m}$ diameter) with excitation and emission wavelengths of 505 and 515, respectively. Red fluorescent microspheres ($1 \mu\text{m}$ diameter) with excitation and emission wavelengths of 580 and 605, respectively. The density of the beads can be adjusted upon request.

Cell Guidance Systems' reagents and services enable control, manipulation and monitoring of the cell, both *in vitro* and *in vivo*.

Growth Factors

- Recombinant
- PODS® Sustained Release

Exosomes

- Purification
- Detection
- Purified Exosomes
- NTA Service

Cytogenetics

- Karyotype Analysis
- Array Hybridization

Other research products

- Matrix Proteins
- Small Molecules
- Cell Counting Reagent
- Lipid Quantification Reagent



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