Frequently Asked Questions (FAQ)

Matrigen Softwell®

Hydrogels

Version 1.0

Contents

General Questions

1. **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

2. **Do you provide smaller pack sizes for initial testing?**

Matrigen products are available for sale as single plates or as packs of 10 dishes/plates.

3. **What is the composition of the gels?**

Softwell® gels 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 gels may be chemically conjugated with ECM ligands to enable cell attachment, growth and differentiation.

4. **Are the gels manufactured under sterile or aseptic conditions?**

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

5. **What is the 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.

6. **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 hydrate, the wells should be filled with PBS until you are ready to use them.

7. **What is the range of available stiffness values?**

Softwell® gels are available in 9 standard stiffness values (0.2, 0.5, 1, 2, 4, 8, 12, 25, and 50 kPa), ultra-soft range (0.03, 0.05, and 0.1 kPa) and screen option (range of standard stiffness values).

8. **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.

9. **Is it possible to have gels with other stiffness values?**

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

10. **What formats are Softwell gels 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.

11. **Which coatings are available in Softwell gels?**

Softwell® gels 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.

12. **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, fibronectin, or laminin.

13. 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.

14. Do you have any experience using gels with other ECM proteins besides Collagen I?

Based on customers' experience, Softwell® gels bind well to different ECM proteins, including fibronectin, laminin, collagen IV, among others. The gels 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.

15. 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.

16. 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.

17. 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: 0.2, 0.5, 1, 2, 4, 8, 12, 25 and 50 kPa, and three wells with no hydrogel. The wells with no hydrogel will have the same treatment of the hydrogels, which can either be Easy Coat™ or Collagen.

Cell Culture and Experimental Questions

18. Are the gels safe for my cells?

Matrigen products are certified non-cytotoxic and pyrogen free.

19. Are the gels compatible with my current assays?

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

20. How do I remove my cells from the gels?

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.

21. Can cells be scraped from the gels?

The cells can be scraped from the gels, but with less mechanical pressure than applied to plastic to avoid disrupting the hydrogel. The softer the gel, the less pressure should be applied. For gels softer than 2 kPa, it is recommended to use a cell scraper with a silicone rubber blade. In this case, the weight of the scraper itself provides sufficient force, so no additional pressure should be applied.

22. How is it recommend to trypsinize cells from the hydrogels?

Matrigen recommends the use of Tryple from ThermoFisher. This reagent will trypsinize the cells even in the presence of serum.

23. Will collagen solubilised in acetic acid be detrimental to the gel?

Acidic solutions will not affect Softwell® gels.

24. 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 gel (200 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.

25. Should I expect auto-fluorescence from Softwell® gels or from antibodies binding to the gels when performing immunofluorescence assays?

Softwell® gels do not have any associated auto-fluorescence and they will not bind to anything besides the ECM proteins used for cell adherence. To avoid any antibodies being retained into the gel, the washing time can be increased. It is also recommended to use 1% BSA and/or 5% goat serum as a blocking buffer to minimize any background in the images.

26. Do Softwell® gels 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).

Quality Control Technical Questions

27. 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/cm^3) upon the surface of the hydrogel. The measurements are taken while the hydrogel and sphere are immersed in phosphate-buffered (PBS) (density = 1 g/cm^3). This procedure is applied to each batch, meaning that each time a stock (prepolymerization) solution of a targeted stiffness is made, an aliquot is polymerized for stiffness validation by using the above described method.

28. 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.

29. 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. The thickness is always >200 µm, which should be sufficient to prevent either individual cells or monolayers of cells from detecting the underlying substrate. On the other hand, the gels 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.

30. What is the size of the pores in the gels?

All the pores in the gels are <0.2 µm. To ensure that this parameter is satisfied, the gels are overlaid with 0.2 µm fluorescent beads to confirm that they cannot penetrate the softest gels.

31. Do you perform electron microscopy to analyze gels morphology?

Matrigen has not performed electron microscopy analysis on the gels.

32. What is the methodology for quality and uniformity control of the collagen coated gels?

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

33. What are SoftTrac hydrogels?

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

34. What will be the use of SoftTrac hydrogels?

SoftTrac™ hydrogels main use is for traction force microscopy.

35. What microspheres are immobilized in the hydrogels?

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