
Silk, Fibroin 50 mg/ml (5%) Solution

Catalog Number: 5154-20ML

Product Description

Advanced BioMatrix's silk solution is approximately 50 mg/mL (5% W/V) of solubilized protein with a molecular weight of approximately 100 kDa available in 20 mL volume. The silk solution is made of 100% silk fibroin protein that is derived from the domesticated *Bombyx mori* silkworm. The product is manufactured in a manner to minimize contamination and has a low bioburden but is not considered sterile.

Fibroin protein is the major structural component of the silkworm's cocoon fiber. Fibroin offers great potential for use in medically related applications due to the high degree of biocompatibility and lack of immune response when implanted within the body. The silk fiber is solubilized into an aqueous fibroin solution, which can then be used as an additive in culture or for producing 3D scaffolds for tissue-engineering related studies.

As with traditional tissue-engineering approaches, the silk scaffolds are typically seeded *in vitro* with a specific cell type as most cells will adhere to fibroin protein, and then cultured over time to mimic tissue architecture. It has been shown that the silk fibroin protein can be degraded by a number of naturally occurring proteolytic enzymes, and is thus a biologically active scaffold unlike other synthetic materials. As a result the silk scaffold material is degraded and remodeled through similar physiological pathways in the body. Silk fibroin protein is composed of both non-essential and essential amino acids, with high concentrations of alanine and glycine, and these amino acids are then reabsorbed by the surrounding cells for new tissue regeneration. This is important as silk degradation products do not collect in the local environment to induce toxicity, which is commonly associated with other synthetic and naturally occurring biomaterials.

The ability to produce a variety of forms and formats (e.g., coatings, films, sponges, hydrogels, electro-spun fibers, micro/nanospheres) offers a number of advantages over other biopolymer systems like collagen, chitosan, and alginate that have less variety in processing choices. The silk material properties can

then be highly modified through a variety of processing techniques to change degradation rate, hydrophobicity/hydrophilicity, transparency, mechanical strength, porosity, oxygen permeability, and thermal stability. In this regard, silk proteins represent a class of engineering biopolymers with definable material properties for a given application.

Characterization

Package Size: 20 mL

Concentration: ~50 mg/mL (5% W/V)

Purity: ≥99% by SDS PAGE Electrophoresis

pH: 8 to 10

Endotoxin: ≤5.0 EU/mL

Bioburden: Low (≤ 10 CFUs) but not sterile

Scaffold Formation: Tested and Passes

Gelation: Tested and Passes

Storage: Store at -70°C.

Thawing/Handling: Thaw slowly at 2 to 10°C. Gently swirl contents to mix. Do not vortex or pipet vigorously. If the entire contents of the 20 mL bottle are not used upon thawing, aliquot into smaller volumes and freeze at -70°C. Minimize freezing and thawing of product. Do not allow product to be thawed for more than week since protein aggregation will likely occur.

Stability/Expiration Date: Stability testing is on-going.

Precautions and Disclaimer

This product is for R&D use only and is not intended for human or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation and Usage

Experimental Protocols for Material Processing:

1. Culture Well Coating Procedure:

Use these recommendations as guidelines to determine the optimal coating conditions for your culture system.

- A. Thaw the product and remove required quantity of silk solution from the bottle and dispense into a dilution vessel.
- B. Dilute silk solution with water to 1 mg/mL (1:50).
- C. Swirl contents gently until material is completely mixed.
- D. Add appropriate amount of diluted silk solution to the culture surface ensuring that the entire surface is coated.
- E. Incubate in a clean bench tissue culture hood at room temperature uncovered for 2-3 hours to allow for complete drying.
- F. After incubation, apply 70% methanol for 20 minutes to induce a water-insoluble silk surface.
- G. Rinse coated surfaces carefully with sterile medium or PBS. Do not scratch surface.
- H. Coated surfaces are ready for use or may be stored at 4°C for future use.

2. Concentrating Silk Solution: This technique is used if a higher silk concentration is desired. Higher silk concentrations may be important for specific processing techniques or to modify final material properties.

- A. Prepare a 10% (W/V) solution of polyethylene glycol (PEG, 10K MW) with deionized water. Mix with a large stir bar on a magnetic stir plate until PEG is completely dissolved.
- B. Obtain a dialysis membrane with a molecular weight cutoff between 3,500 and 10,000 Da. If necessary hydrate the dialysis cassette per the manufacturer requirements.
- C. Fill the dialysis membrane with the appropriate amount of 5% silk solution per the dialysis

membrane manufacturer guidelines for filling volume.

- D. Place the silk solution filled dialysis membrane into the 10% (W/V) PEG solution and cover.
- E. Indicate the time and date that the cassette was added to solution. Typical concentrating times will vary depending on the desired final concentration of silk solution, the volume of silk solution being concentrated, and the dialysis membrane used.

Note: As a typical example, 10 mL of 5% silk solution will yield 2–4 mL of concentrated silk solution after dialyzing against 10% (W/V) PEG for 20–22 hours. In general, optimization runs are usually recommended per user requirements.

- F. Remove the concentrated silk solution from the PEG bath and dialysis cassette after the concentrating time has finished and store silk solution at 4°C for future use. **NOTE:** Silk solution shelf life decreases with increasing concentration, so use concentrated silk solution soon within 1 week after it is produced.
- G. Silk solution concentration can be determined by weight percent concentration. To do this, weigh out approximately 100 µl of concentrated silk solution on a precision balance and record the wet weight. Allow the solution to dry into a film and measure the silk protein dry weight. Divide the dry weight over the wet weight and multiply by 100% to get the weight percent concentration of the solution.

3. Freestanding Silk Films: This processing method produces freestanding silk film materials that can be used for cell culture or *in vivo* transplantation. Freestanding silk films offer the advantage of easy removal from culture conditions for further sample analysis.

- A. Add 7 mL of 5% silk solution into a 100 mm Petri dish and allow drying uncovered in a clean bench environment. This typically takes several hours and is best left overnight.
- B. The formed film will be approximately 40-60 µm in thickness and can be removed from the Petri dish using forceps. Increasing the amount of silk solution or using higher concentration silk solution produces thicker films.

- C. The films are currently water-soluble and can be made insoluble for cell culture using either of the following methods:

- i. Methanol bath incubation:
 - a. Fill dish with 70% methanol and 30% deionized water and mix.
 - b. Place silk film into methanol solution for 10 minutes.
 - c. Remove silk film and rinse with sterile water or appropriate medium.

Note: This processing method rapidly produces insoluble silk film material properties that tend to be opaque, more hydrophobic, and have slow degradation rates *in situ*.

- ii. Water-annealing:
 - a. Obtain an emptied lab vacuum desiccator and fill bottom partially with water.
 - b. Place films on shelf above water, cover, and then pull a 25 in Hg vacuum.
 - c. Stop cock the chamber and allow to sit for 4 hours.
 - d. Remove samples from chamber and sterilize with 70% ethanol and then rinse with sterile water or appropriate media.

Note: this processing method produces insoluble silk film material properties that tend to be transparent, more hydrophilic, and have fast degradation rates *in situ*.

- D. Films can then be cut to shape and stored for 2 years or more at room temperature if not used immediately.

Note: Silk films can also be cast onto patterned surfaces to replicate the surface topography, typically silicone rubber or similar materials will be used for ease of silk material removal. In addition, weighting methods may be required to keep films submerged in media due to potential material buoyancy.

4. 3D Silk Sponge Scaffolds: This processing method creates 3D porous scaffolds for both *in vitro* and *in vivo* applications or *in situ* implantation. The scaffold pore size and degradability characteristics can also be tuned using the method below.

- A. Aliquot silk solution into a desired molding vessel, Teflon® is recommended to allow for ease of material removal.
- B. For each sample being prepared weigh the necessary amount of salt to maintain a 25:1 ratio of salt to silk weight.

Note: The control over pore size is dictated by the chosen sodium chloride (salt) crystal size. If a defined pore size is preferred use stainless steel sieves to produce a uniform salt crystal size. It is recommended for salt particles that are 750 µm and larger that the silk solution is concentrated to 8% or above.
- C. Add the salt slowly to the silk solution while rotating the molds to allow for uniform salt addition. Be sure to carefully remove air bubbles formed from material displacement through mild agitation or tapping on a surface.
- D. Cover the mold and allow the solution to sit for 1-2 days at room temperature to allow for silk scaffold formation.
- E. After the scaffold has formed remove the mold cover and place samples into a 2-liter beaker of DI water and place on stir plate.
- F. Change the water every 8-12 hours for 48 hours.
- G. After the washing period remove the formed scaffolds from the mold and place in a final deionized water rinse for 24 hours to ensure complete removal of residual salt.
- H. Scaffolds can be stored in deionized water for at 4 °C or dry at room temperature until needed.
- I. The scaffolds can be cut to the required dimensions and steam sterilized before using.

5. 3D Silk Hydrogels: This processing method can be used to produce silk hydrogels for use as an injectable biomaterial, *in vitro* culture, and *in vivo* use.

- A. Place the 5% silk solution into the desired molding vessel or vial.
- B. Secure the vessel or vial to a lab vortexer with tape in an upright position.
- C. Vortex the solution for a several minutes and at maximal rotational speed. These parameters will need to be optimized for the given silk solution volume and mold geometry.

Note: As an example, pipette 1 mL of silk solution in a glass vial and vortex for ~7 min at 3,200 RPM. The solution should increase in turbidity indicating the gelation process has

been initiated.

Note: Once the solution has been vortexed, do not transfer the solution to a secondary container before gelation has completed.

- D. Place the vortexed silk/molding vessel/vial in a 37 °C incubator overnight to expedite gelation time.
- E. Silk hydrogels can be used for future use as long as they remain hydrated and stored in deionized water at 4 °C.

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