Fibrothelium

Instructions for Use

PureSilk®
fibroin solution

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Product Name: PureSilk® fibroin solution

Product Description

PureSilk® fibroin solution is an aqueous protein solution with solubilized silk fibroin protein available in volumes of 30 mL and 250 mL. PureSilk® fibroin solution contains silk fibroin protein that is derived from the cocoons of the domesticated Bombyx mori silkworm. PureSilk® fibroin solution is obtained by harvesting silk fibroin with an innovative procedure (patent pending). This process is highly effective and environmentally friendly since it avoids the use of potentially harmful solvents traditionally used in silk fibroin isolation. The high quality of this solution is achieved through a manufacturing process aimed at minimizing the risk of contamination as certified by the low content in endotoxins and CFU during tests. The solution is not considered sterile.

From PureSilk® fibroin solution it is possible to create a variety of shapes and structures (e.g., scaffolds, membranes, coatings, sponges, hydrogels etc.) with tuneable mechanical and structural properties, offering many potentialities. Moreover, the silk proteins contained in the solution can be modified through a variety of processing techniques to change degradation behavior, hydrophobicity/hydrophilicity, optical abilities, mechanical properties, porosity, permeability, bioactivity and thermal/chemical stability. In this regard, silk proteins represent a class of engineering biopolymers with highly adjustable material properties suitable for any given application.

Preparation and Usage

Storage/Expiration Date:	Store at -20 °C, expiration after 90 days (without thawing).
Thawing/Handling:	Thaw in a warm water bath at 30 to 35 °C. Gently swirl contents to mix. Do not vortex or pipet vigorously. If the entire content of the bottle is not used upon thawing, aliquot into smaller volumes and freeze. Minimize freezing and thawing of the product. Do not allow the product to be stored at temperatures above -20 °C for more than 3 days since protein aggregation will likely occur.
Centrifugation:	Before using the solution after thawing, it is recommended to centrifuge it in a sterile vessel at 2,000 RCF for at least 15 min to bring down possible protein flocculation. Pour off the supernatant for further use.
Concentrating:	Use one of the following techniques to increase the concentration of your PureSilk® fibroin solution, which may be necessary depending on the following process. 1) Concentrate by use of polyethylene glycol (PEG 10-20 kDa MW) a. Prepare a PEG solution with at least 10 % (w/v) (higher concentrations enable faster process, below 5 % the process becomes ineffective) b. Fill a dialysis system (molecular cutoff < 10 kDa) with fibroin solution (follow the guidelines of the dialysis system manufacturer) c. Place the filled dialysis system into the PEG solution, cover the vessel and stir the solution slowly. d. Depending on the desired fibroin concentration, the used PEG concentration and the amounts of both, the process can last a few days Note: 100 mL Fibroin 3.5 % → 15 % with 1000 mL 15 % PEG ≈ 24 h Optimization runs are usually recommended per user requirements Conductivity can be used to observe the process by running a %-PEG-Conductivity Ratio and relate it to the dialysis system deprived water e. After reaching the desired concentration, it is advised to put the filled dialysis system into distilled water for at least 3 h again, to remove remaining ethylene glycol monomers f. Remove the concentrated silk solution from the bath and store it at < 4 °C for future use Note: PureSilk® fibroin solution shelf life decreases with increasing concentration, so use soon within one week after it is concentrated

Concentrated solutions become more susceptible for gelation induced by impact, so handle them with care

PureSilk® fibroin solution concentration can be determined by weight percent. To do this, weigh out approximately 100 µl of concentrated PureSilk® fibroin solution on a precision balance and record the wet weight. Allow the solution to dry and measure the dry weight. Calculate the concentration as follows:

$$w/w\ \% = \frac{m_D}{m_w} \cdot 100\ \cdot \%$$

m_D = Dry weight; m_W = Wet weight

2) Concentrate by heat

a. Prepare an appropriate glass vessel (the more surface for the solution, the faster the process) with a magnetic stirrer for the solution

Note:

The stirrer should be fully covered by solution

b. Heat the solution under constant stirring at max. 50 °C (higher temperatures may lead to denaturation, followed by gelation)

Note:

The stirring needs to stir up the surface to prevent film formation, but not too fast to prevent foam formation

c. Concentrate till the desired volume, then let the solution cool down at room temperature under constant stirring

d. Store it at < 4 °C for future use.

Note:

Silk solution shelf life decreases with increasing concentration, so use soon within one week after it is concentrated

Concentrated solutions become more susceptible for gelation induced by impact, so handle them with care

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 and measure the dry weight. Calculate the concentration as follows:

$$w/w\ \% = \frac{m_D}{m_w} \cdot 100$$

m_D = Dry weight; m_W = Wet weight

An overview of different ways of processing fibroin solution are given in the following article:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3808976/

DOI:10.1038/nprot.2011.379

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