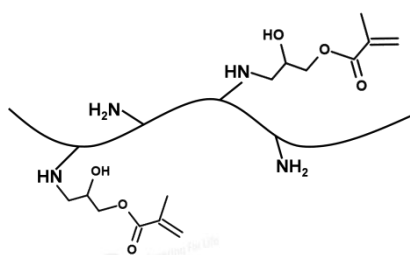


Silk Fibroin Methacryloyl (SiIMA)

Product component

Item	Character	Package Size	Notes
A: SiIMA	White spongy	1g/bottle	Keep in dark
B: Photoinitiator LAP	White powder	0.05g/bottle	

This instruction applies to EFL-SiIMA-001



SiIMA molecular structure

Product introduction

Silk fibroin (SF) is derived from the degumming of silk, and is a polypeptide composed of a variety of amino acids. SF molecule includes a hydrophobic peptide chain (H chain) and a hydrophilic peptide chain (L chain). The special amino acid sequences of H chain and L chain make it form a variety of protein secondary conformations. The properties of silk fibroin materials can be effectively controlled by regulating the secondary structure of silk fibroin, including the preparation of high strength and high orientation materials. SF has good biocompatibility, biodegradability and high tensile strength. It has been used in various biomedical fields, including wound dressings, artificial blood vessels, cell culture, etc.

Silk fibroin methacryloyl (SiIMA) is modified by methacryloyl modification of SF by glycidyl methacrylate to introduce double bonds on SF molecules. Due to the special spatial structure of SF molecule, it is easy to form crystallization and insoluble in water before modification. After the introduction of additional chemical groups, it can be quickly dissolved in water, which enables SiIMA to be photocurable into hydrogels.

Storage

Dry kit: room temperature, 3 months; 4°C, 12 months; -20°C, 18 months. **It is best to prepare it when using it.**



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Period of validity

The date of manufacture is shown in the package.

Solution preparation

Step1. Prepare the initiator standard solution (0.25%(w/v), 2.5mg/ml. It is recommended to use EFL's photoinitiators available for sale: Model EFL-LAP)

Add the required LAP to PBS solution and dissolve in a water bath at 40-50°C to obtain 0.25% (w/v) initiator standard solution.

Step2. Prepare the SilMA solution (It is recommended that the SilMA concentration be 8-20%(w/v), 80-200mg/ml)

- (1) Take the required mass of SilMA into the centrifugal tube;
- (2) Add the initiator standard solution into the centrifuge tube;
- (3) Dissolve at room temperature for 0.5-1h, stirring / oscillating several times (Avoid intense ultrasound, high temperature and strong shearing);
- (4) Sterilize the SilMA solution immediately with a 0.22μm sterile needle filter.

Note: Stir/Shake immediately after adding the solvent. Ensure that the sample is in full contact with the solvent and dispersed in the solvent to avoid deformation. They form lumps that are difficult to dissolve and "wet on the outside and dry on the inside". Silk fibroin solution is a semi-steady-state sol, which is easily induced by external stimuli such as strong shear, ultrasound, high temperature, organic solvents, etc, and molecular self-assembly occurs, precipitation or gelation behavior occurs. It is best to prepare it when using it. A small amount of insoluble material in the silk fibroin solution is normal. It is recommended to remove insolubles by centrifugation or filtration to avoid inducing more silk fibroin precipitation.

Suggestions for 2D cell culture

- Inject SilMA solution into the well plate;
(96-well plate: 50-100μL/ well, 48-well plate: 100-300μL/ well, 24-well plate: 300-500μL/ well)
- Irradiate the wells with 405nm light for 10-30 seconds to gelate, the gel strength can be adjusted by the time and intensity of the light;
- Add medium to the wells to cover the gel. Place the well plate in a 37°C incubator for 5 minutes. And then wash the sample and remove the medium;
- Add the cell suspension to the well plate. Change medium, observe, and photograph according to experimental design. (No special requirements for



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operation procedures).

(Suggestion for promoting cell adhesion culture : SilMA hydrogel was soaked overnight in medium or 75 % ethanol to produce hydrophobic crystalline microdomains to promote cell adhesion.)

Suggestions for 3D cell culture

- Cells were collected and resuspended in SilMA to prepare the cell suspension;
- Add cell suspension into the well plates;
(96-well plate: 50-100 μ L/ well, 48-well plate: 100-300 μ L/ well, 24-well plate: 300-500 μ L/ well)
- Irradiate the wells with 405nm light for 10-30 seconds to gelate, the gel strength can be adjusted by the time and intensity of the light;
- Add medium to the wells. Place the plate in a 37°C incubator for 5 minutes. And then wash the sample and remove the medium;
- Add fresh medium and incubate for a long time. Change medium, observe, and photograph according to experimental design. (No special requirements for operation procedures).

Tips: Do not look directly at the light source.



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