

## Directions for Use

### PhotoChitosan®-RUT Methacrylated Chitosan Kit

CHITOSAN METHACRYLATE WITH RUTHENIUM KIT FOR PHOTOCROSSLINKABLE  
HYDROGELS

Catalog Number **#5442-1KIT**

#### Product Description

Advanced BioMatrix offers PhotoChitosan®, a sterile chitosan methacrylate kit that provides customers with a tunable chitosan-based bioink. 3D chitosan gels can be prepared at various concentrations and photocrosslinked to provide different gel stiffnesses.

The PhotoChitosan® RUT kit consists of Chitosan methacrylate and a visible light photoinitiator.

Table 1:

Item	Catalog No.	Package Size
Methacrylated Chitosan, Lyophilized	5428-100MG	100 mg
Photoinitiator Ruthenium	5246-100MG	100 mg
Photoinitiator Sodium Persulfate	5247-500MG	500 mg

Our Chitosan Methacrylate achieves a degree of substitution 2-15% for maximum crosslinking and range of stiffness.

The photoinitiator solution consists of Ruthenium and Sodium Persulfate which needs to be formulated in 1X PBS, allowing for visible light photocrosslinking of the sericin at 400-450 nm.

#### Storage/Stability:

The product ships on frozen gel packs. Upon receipt, store the chitosan methacrylate at -20°C. Store the ruthenium and sodium persulfate at room temperature.

#### Preparation Instructions

PhotoChitosan is soluble in aqueous 20 mM acetic acid. For cell culture applications, 1X PBS is recommended for dissolution.

Note: Employ aseptic practices to maintain the sterility of the product throughout the preparation and handling of the chitosan and other solutions.

Note: The following instructions are for a 1% chitosan methacrylate solution. Recommended working concentrations are 0.5 - 1%

1. Add the 10 mL's of 1X PBS to the amber vial containing 100 mg of lyophilized chitosan methacrylate. The resulting pH should be ~6, and can be increased with addition of sterile 0.1M NaOH (order of microliters).
2. Gently mix on a shaker table or stir plate overnight or until fully solubilized. Avoid vortexing and shaking the bottle to prevent unsolubilized chitosan from sticking to the sides of the vial. Mixing may be done warm (up to ~65°C) or at room temperature.
3. Calculate the volume of Ruthenium and Sodium Persulfate to add by multiplying the volume of solubilized chitosan by 0.02. If the resulting number is 200 µl, for example, you will add 200 µl of Ruthenium and 200 µl of Sodium Persulfate.
4. Solubilize the required amount of ruthenium (per step 3) at a concentration of 37.4 mg/ml in

1X PBS or cell culture media. Photoinitiator can be sterilized through a 0.2um button filter.

5. Solubilize the required amount of Sodium Persulfate (per set 3) at a concentration of 119 mg/ml in 1X PBS. Photoinitiator can be sterilized through a 0.2  $\mu$ m button filter.
6. Add the Ruthenium to the chitosan solution and fully mix until solution is homogeneous.
7. Add the Sodium Persulfate to the PhotoChitosan/Ruthenium solution and mix until the solution is homogeneous.
8. Dispense your bioink solution into a well plate or petri dish for photocrosslinking or use for bioprinting at 400-450 nm

Any excess material can be refrigerated and stored. Long term shelf-life studies are in process. We recommend only adding photoinitiator to the amount of PhotoChitosan to be used at that time.

