Directions for Use

Ruthenium Visible Light Photocrosslinking Kit
for 3D hydrogels or bioinks

Catalog Number #5248-1KIT

Product Description

The Ruthenium visible light photocrosslinking kit is ideal for tissue engineering, cell culture, and bioprinting, where tuning the mechanical properties of the substrate is required. The kit provides enough photoinitiator for >200 mL of bioinks/hydrogels (following recommended concentrations).

Ruthenium is a photoinitiator that utilizes visible light photocrosslinking (400-450nm) to covalently crosslink free tyrosine and acryl groups.

Ruthenium photoinitiator has been tested on collagen type I, gelatin, silk fibroin, methacrylated hyaluronic acid, methacrylated gelatin, methacrylated collagen type I and PEGDA.

The Ruthenium is water soluble and yields better cell cytocompatibility, and crosslinking efficiency.

Increasing Visible light intensity from 3-100 mW/cm2 using Ruthenium did not significantly decrease cell viability from 90%.

Increasing Ruthenium concentration by 10 times (10x) did not decrease cell viability from 90%.

Ruthenium is red/yellow/orange in color and can change the color of your solutions, hydrogels, or printed constructs.

This Ruthenium photocrosslinking kit is considered non-sterile. Adding antibiotics to your cell culture system, or sterile filtering is recommended. To sterile filter, resuspend the entire volume of Ruthenium and Sodium Persulfate **(separately)** and filter through small 0.2 micron button filters **(separately)**. Use the sterile photoinitiator within 2 weeks.

The Ruthenium visible light photocrosslinking kit is composed of two components, as found in Table 1.

Table 1:

Item	Catalog No.	Package Size
Ruthenium Powder	5246-200MG	200 mg
Sodium Persulfate Powder	5247-1GM	1 gram

Storage/Stability:

The product ships ambient. Store the kit at room temperature. Weigh out the required amount of powder to solubilize. Once solubilized, use the Ruthenium and Sodium Persulfate within 2 weeks.

Dry powder (non-solubilized) is stable for >1 year at room temperature.

Preparation Instructions
(Example calculations can be found at the bottom of the document)

1. Calculate desired volume of hydrogel or bioink (ECM + cells).

2. Multiply desired volume by 0.02. This is how much Ruthenium and Sodium Persulfate (each) you will be adding to your pre-hydrogel solution.

3. Solubilize required Ruthenium in water or 1X PBS at a concentration of 37.4 mg/mL.

4. Solubilize Sodium Persulfate in water or 1X PBS at a concentration of 119 mg/mL.

5. Add the calculated volume (step 2) of Ruthenium to your pre-hydrogel solution and thoroughly mix.

6. Add the calculated volume (step 2) of Sodium Persulfate to your pre-hyrogel solution and thoroughly mix.

Notes:
Do not mix the Ruthenium and Sodium Persulfate together prior to adding to the pre-hydrogel. You will get a rapid redox reaction and they will precipitate instantaneously.

7. Add in cells, if desired.

8. Photocrosslink at 400-450nm wavelength. Initial recommendation is 50 mW/cm2 for >3 minutes to maintain shape/hydrogel fidelity. You may tune photoinitiator concentration, light intensity and Photocrosslinking time to customize final hydrogel stiffness.

Additional Notes:

If using neutralized type I collagen, you can allow the collagen to polymerize at 37C to form a hydrogel, and then photocrosslink to further crosslink and modulate the gel stiffness.

How to use Ruthenium with Lifeink® 200 3D Bioprinting:

1. Print Lifeink® 200 according to the recommended protocols, into the FRESH support slurry.

2. After printing, incubate the print to melt the FRESH gelatin support slurry.

3. After ~30 minutes, replace the melted gelatin with warm cell culture media.
4. For example, pipette out 2 mL of melted gelatin, and then add in 2 mL of media. Repeat until the gelatin is removed.

5. Prepare stock solutions of Ruthenium and Sodium Persulfate (found in the above protocol).

6. Multiply total volume of media that your Lifeink® 200 structure is floating in. Multiply by 2%, and add that amount of Ruthenium and Sodium Persulfate to the cell culture media (directly in the same dish that your structure is in).

7. Photocrosslink with visible light (400-450 nm) until desired crosslinking is achieved.

8. Gently replace the media with fresh media, as done in step 3.

Example Calculations:

To photocrosslink 20 mL of neutralized collagen:

Required volume of Ruthenium/Sodium Persulfate is 20 mL x 0.02 = 0.4 mL

Ruthenium to add = 0.4 mL x 37.4 mg/mL = 14.96 mg.
Solubilize 14.96 mg of Ruthenium in 0.4 mL of 1X PBS or water.

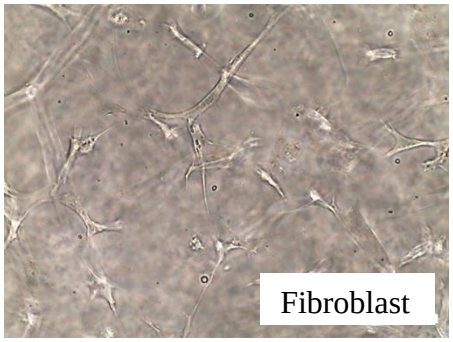
Sodium Persulfate to add = 0.4 mL x 119 mg/ml = 47.6 mg.
Solubilize 47.6 mg of Sodium Persulfate in 0.4 mL of 1X PBS or water.

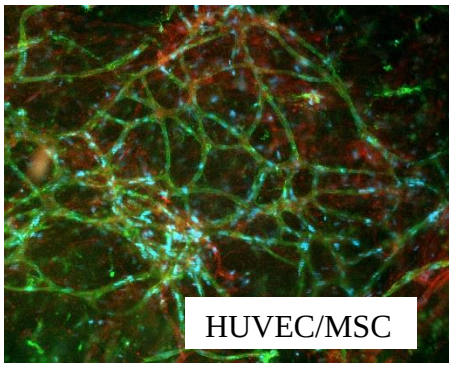
Follow the above directions for mixing in the photoinitiators with the collagen solution.

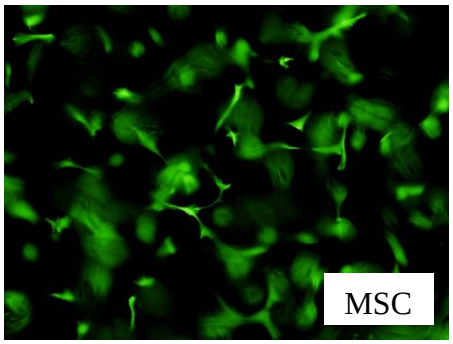
Reference:
Lim, Khoon S., et al. "New visible-light photoinitiating system for improved print fidelity in gelatin-based bioinks." *ACS Biomaterials Science & Engineering* 2.10 (2016): 1752-1762.

Supplemental Material
Ruthenium has been tested with the following cells:

Human mesenchymal stromal cells (hMSC)
Human articular chondrocytes (HAC)
Human nasal chondrocytes (HNC)
Human umbilical vein endothelial cells (HUVEC)
Human endothelial colony forming cells (ECFC)
Human neonatal fibroblasts (HNF)
Human breast adenocarcinoma cells (MCF7, MDA-MB-231)
Human breast ductal carcinoma cells (HCC1954)
Human ovarian adenocarcinoma cells (SKOV3)
Human mature adipocytes
Human adipose derived stem cells (hASC)
Human annulus fibrosus cells (HAF)
Human induced neural progenitor cells
Bovine articular chondrocytes
Equine articular chondrocytes
Equine chondroprogenitor cells
Porcine articular chondrocytes
Murine cardiac myocytes (HL1)
Murine pheochromocytoma cells (PC12)
Murine teratocarcinoma cells (ATDC5)
Murine breast adenocarcinoma cells (EO771)







Images and cell studies provided and performed by University of Otago