

# Regenix Powder Protocol



Regenix is composed of various extracellular matrix proteins derived from native tissues. Provided in powder form, it can be utilized not only for 2D and 3D cell culture but also for a wide range of tissue engineering applications. This versatility allows for the creation of an optimized environment for cell growth and regeneration across various biomedical fields.

## Storage Instructions

- ⌚ Regenix powder can be stored refrigerated or frozen, with freezing recommended for long-term storage.
- ⌚ Avoid exposing Regenix powder to frequent temperature changes, as the powder may absorb moisture.
- ⌚ If planning for multiple uses, aliquot the powder immediately upon receipt to prevent moisture absorption.

## Regenix Solution Preparation Instructions

- ⌚ Regenix solutions (10 mg/mL) can be prepared by enzymatic digestion of Regenix powder using pepsin (2–8 mg/mL).
- ⌚ The pH of the solution should be optimized with HCl (0.01–0.02 M) according to the experimental purpose.
- ⌚ The time required for Regenix powder to fully dissolve varies depending on the experimental conditions, but it generally takes about 48 hours.

## Pre-Gel Solution Preparation Instructions

- ⌚ The pre-gel solution can be prepared by mixing the Regenix solution with 10% (v/v) 10× phosphate-buffered saline (PBS).
- ⌚ The pH of the pre-gel solution should be adjusted to 7.0–7.4 by gradually adding sodium hydroxide (NaOH). Since crosslinking may begin at room temperature, the preparation of the pre-gel solution should be done on ice (4°C).
- ⌚ To fabricate the Regenix hydrogel, incubate the pre-gel solution at 37°C for 40 minutes to allow gelation.

## Surface Coating Instructions

- ⊙ Both the pepsin-solubilized Regenix solution and the fabricated pre-gel solution can be used for surface coating on glass or plastic substrates.
- ⊙ When preparing the Regenix solution at a concentration of 2 mg/mL, a dilution ratio of 1:20 to 1:40 is recommended. Poly-L-lysine (PLL) pre-coating can enhance the efficiency of the coating, allowing for the use of smaller amounts of Regenix solution.
- ⊙ For dilution, we recommend using pre-chilled basal medium (without supplements), PBS, 0.01–0.02 M HCl solution, or 0.02 M acetic acid solution.
- ⊙ Always keep the Regenix solution on ice during the coating process to prevent premature gelation.
- ⊙ Incubate the coated surface at 37°C for 2 hours to ensure complete surface coating
- ⊙ After incubation, aspirate any remaining Regenix solution and rinse the surface with PBS or basal medium.