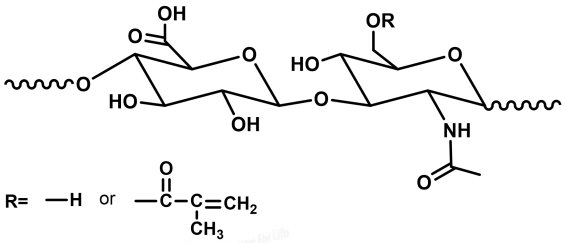
# Hyaluronnic acid Methacryloyl(HAMA)

## Product component

| Item | Character | Package Size | Notes |
|---|---|---|---|
| A: HAMA | White spongy | 0.2 or 0.5g/bottle | Keep in dark |
| B: Photoinitiator LAP | White powder | 0.025g/bottle | |

This instruction applies to EFL-HAMA-150K/400K




**HAMA molecular structure**

## Product introduction

Hyaluronic acid (HA) is a natural glycosaminoglycan polymer consisting of D-glucuronic acid and N-Acetyl-D-Glucosamine as disaccharide structural units. It is a component of the extracellular matrix of animal tissues and has good moisture retention properties. Its content is high in brain tissue, synovial fluid, and vitreous body. HA plays an important role in many biological processes such as cell proliferation, differentiation, morphogenesis, inflammation, and wound healing.

Hyaluronic acid Methacryloyl(HAMA)can be photo-crosslinkedby grafting methacryloyl group on the molecular chain of hyaluronic acid. The HAMA product launched by the EFL team can be cured into gel within 10 seconds under visible light irradiation. It has good biocompatibility, and strong material scalability, and can provide a variety of viscoelastic properties to adapt to different applications.

## Storage

**Dry kit**: room temperature, 3 months; 4℃, 12 months; -20℃, 18 months. **Sterile solution**:4°C (in dark),7 days;-20 ℃ (in dark),6 months. **Please note that repeated freezing and thawing of the solution will affect the performance of the product, so it is best to prepare it when using it.**

## 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)**

(1) Add 10ml PBS into the brown bottle containing initiator LAP (containing 0.025g LAP);

(2) Heat and dissolve the solution in a water bath at 40-50℃ for 15 minutes, shaking several times.

The LAP standard solution can be stored for 12 months at 4°C in dark.

**Step2. Prepare the HAMA solution (It is recommended that the concentration of HAMA-150K be 2-10% (w/v), 20-100mg/ml. The concentration of HAMA-400K is 0.5-3% (w/v), 5-30mg/ml)**

(1) Take the required mass of HAMA into the glass bottle/beaker;

(2) Take the required volume of initiator standard solution and add it to the above container;

(3) Stir and dissolve at room temperature for 0.5-1h;

- The viscosity of the HAMA-400K solution is large. The dissolution time can be extended appropriately. Pay attention to seal to prevent moisture volatilization;
- It is recommended to use centrifugal machines (3000~5000rpm, 2-3min) to remove bubbles from the system;

(4) Solution sterilization.

- Method 1: Sterilize with 0.22μm sterile needle filter;
- Method 2: Pasteurization: The solution was heated to 80℃ and held for 30min. Transfer quickly to the ice-water mixture solution for 5 minutes. Repeat the above operation once again.

## Suggestions for 2D cell culture

- Inject the HAMA solution into the well plate;
  (96-well plate: 50-100μL/ well, 48-well plate: 100-300μL/ well, 24-well plate: 300-500μL/ well)
- 405nm light source is used to irradiate for 10-30 seconds to gelate. The gel strength can be regulated by the duration and concentration of illumination.
- Add the culture medium to the Wells, cover them with gel, place them in an

incubator at 37 ℃ for 5 minutes, clean the samples, and aspirate the culture medium.

- Just add the cell suspension to the well plate. Carry out operations such as medium replacement, observation and photography according to the experimental design.(There are no special requirements for the procedure.)

## Suggestions for 3D cell culture

- Cells are collected and resuspended in pre-warmed HAMA solution to prepare the cell suspension;
- Add cell suspension into the well plates;
  (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. Place the plate in a 37℃ 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.**