PEGSSDA
THIOL-REACTIVE CROSSLINKER, 0.5 ML
Catalog Number **#GS755**

OVERVIEW
PEGSSDA™ (PEGSSDA, disulfide-containing polyethylene glycol diacrylate) is packaged in 0.5 mL aliquots. Vials are blanketed by nitrogen and under a slight vacuum.

STORAGE
Store PEGSSDA in the original vial unopened at -20°C for up to one year. Reconstituted solutions can be stored at -20°C for ~ one month.

INSTRUCTIONS FOR USE
PEGSSDA is prepared by dissolving the lyophilized solid in the DG Water. When reconstituted, it will be in 1x phosphate buff-ered saline (PBS) buffer pH ~7.4.

PEGSSDA should be prepared in the following manner:

1. Allow the PEGSSDA vial to come to room temperature.
2. Under aseptic conditions using a syringe and needle add to the vial the 0.5 mL of DG Water as indicated on the label.
3. Invert several times to dissolve.
4. PEGSSDA is used to chemically crosslink hydrogels made from Glycosil, Heprasil, and Gelin-S. PEGSSDA does not form a hydrogel on its own.
5. Typically PEGSSDA is used in a 4:1 volume ratio with Glycosil, Heprasil, and Gelin-S, as follows:
    a. 0.25 mL PEGSSDA is crosslinked with 1.0 mL Glycosil
    b. 0.25 mL PEGSSDA is crosslinked with 0.5 mL Glycosil
    + 0.5 mL Gelin-S
    c. 0.25 mL PEGSSDA is crosslinked with 1.0 mL Heprasil
    d. 0.25 mL PEGSSDA is crosslinked with 0.5 mL Heprasil
    + 0.5 mL Gelin-S
6. Gelation time varies depending upon the amount of PEGSSDA, the amount of Glycosil or Heprasil, and the amount of Gelin-S used. Hydrogels that include Gelin-S will typically have longer gelation times than those made with only Glycosil or Heprasil.
7. Note: Gelin-S will not form a hydrogel when mixed with PEGSSDA.

Note: Hydrogels made using only PEGSSDA and Glycosil or Heprasil will not support cell attachment.

DISSOLUTION
Dissolution of gels with cells on top and encapsulated (gel volume of 0.6 mL) in a 24-well plate. The following procedure was optimized particularly for the aforementioned gel geometry. Dissolution of gels with alternate geometry and/or volumes may require adjustments to the protocol.

1. Make up the appropriate amount of 40mM N-Acetyl-L-Cysteine in 1X PBS or media and pH to 7.4.
2. Add 2 mL of 40mM N-Acetyl-Cysteine to the top of each gel and let sit at 37°C for 0.5 hours. Agitation by orbital shaking will help decrease dissolution time.
3. After 0.5 hours mix the gel and N-Acetyl-L-Cysteine together by pipetting up and down with a 1 mL pipet until there is no longer any resistance upon filling the pipet tip.
4. Let incubate at 37°C for another 0.5 hours.
5. Mix again as in step 3.
6. Repeat this process for a total time of 2h from the first addition a N-Acetyl-LCysteine.

7. Remove liquid from well and place in conical centrifuge tube. Add PBS to a total of 5mL of liquid.
8. Centrifuge at 1000 RPM for 5 minutes.
9. Aspirate off PBS and process cells as desired.

Note: Each kit component has been manufactured under aseptic conditions and tested for bacteria and fungus.