



## HyStem-C and HyStem-HP Gelation Time Variation

The HyStem<sup>®</sup>-C Hydrogel Kit is composed of Glycosil<sup>®</sup> (CMHA-S, thiol-modified hyaluronic acid), Gelin-S<sup>®</sup> (Gtn-DTPH, thiol-modified gelatin), Extralink<sup>®</sup> (PEGDA, polyethylene glycol diacrylate), and degassed, deionized water (DG Water).

The HyStem-HP Hydrogel Kit has the same composition except that Glycosil is replaced with Heprasil<sup>®</sup> (CMHA-S, thiol-modified hyaluronic acid with thiol-modified heparin). Solutions of Glycosil or Heprasil and Gelin-S form a transparent hydrogel when mixed with Extralink. The standard formulation assumes that Extralink will be added in a 1:4 volume ratio to an equal volume mixture of Glycosil/Heprasil + Gelin-S. The resulting gelation time will be ~20 minutes. The gelation time can be varied by changing the following parameters:

- pH of Glycosil + Gelin-S solution
- Ratio of Glycosil to Gelin-S (more Glycosil = faster gelation)
- Ratio of Extralink to Glycosil + Gelin-S (more Extralink = faster gelation)
- Amount of liquid used to resuspend Extralink (more concentrated Extralink = faster gelation)

Below is data demonstrating how the gelation time varies with different conditions. All data is given for hydrogels made with Glycosil but applies equally to those made with Heprasil. If the gelation time is critical for your experiment, we recommend that a bench top gelation test be conducted prior to using the hydrogels for in vitro or in vivo experiments.

The gelation time is very pH sensitive – the higher the pH, the faster the gelation time. The data presented is for Glycosil only hydrogels. However, the same trend holds for Gelin-S containing hydrogels.

Glycosil, mL	Extralink, mL	pH	Gelation, min
0.5	0.125	6.76	0:50
0.5	0.125	7.07	0:32
0.5	0.125	7.28	0:23
0.5	0.125	7.60	0:12
0.5	0.125	8.59	0:02