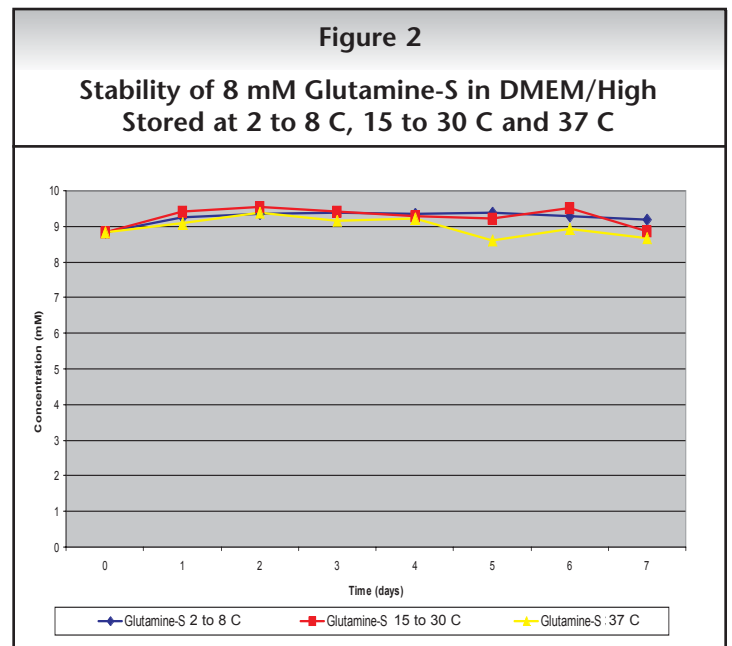
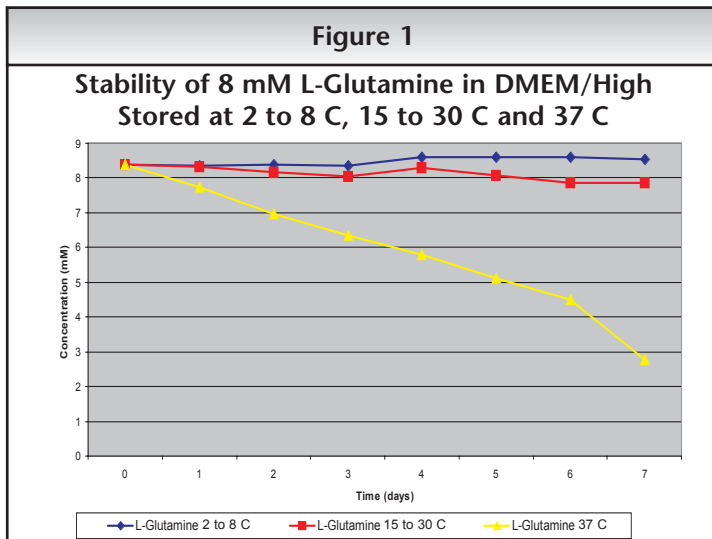


Technical Bulletin

Glutamine-S Stability

SAFC Biosciences provides a stabilized dipeptide form of L-alanyl-L-glutamine, Glutamine-S 200 mM, which postpones the degradation of L-glutamine, thereby prolonging supplemental degradation that could compromise cell culture longevity. This unique dipeptide structure not only resists degradation in medium, but prevents the accumulation of cytotoxic ammonia because of its enhanced stability.

An investigation using Glutamine-S 200 mM (Catalog No. 59212) and L-Glutamine Solution 200 mM (Catalog No. 59202) supplemented at 8 mM in Dulbecco's Modified Eagle's Medium/High Modified (DMEM/High) (Catalog No. 51440) demonstrated the consistent stability of the dipeptide under various storage conditions as compared to that of L-glutamine (see Figures 1 and 2).



The stability of Glutamine-S in medium over time is important in demonstrating this product's advantages over normal L-glutamine supplementation. The use of Glutamine-S, aimed at maintaining consistent levels of glutamine during 2 to 8 C storage of supplemented medium, can be applied further to the product's stability under culturing conditions. An investigation looked at L-glutamine and Glutamine-S at 8 mM concentrations in DMEM/High and demonstrated the degradation trends when stored at 37 C. Samples were taken daily for seven days and analyzed for the remaining levels of their respective glutamine formulations in order to observe the stability of each solution when simulating cell culture incubation (see Figure 3).

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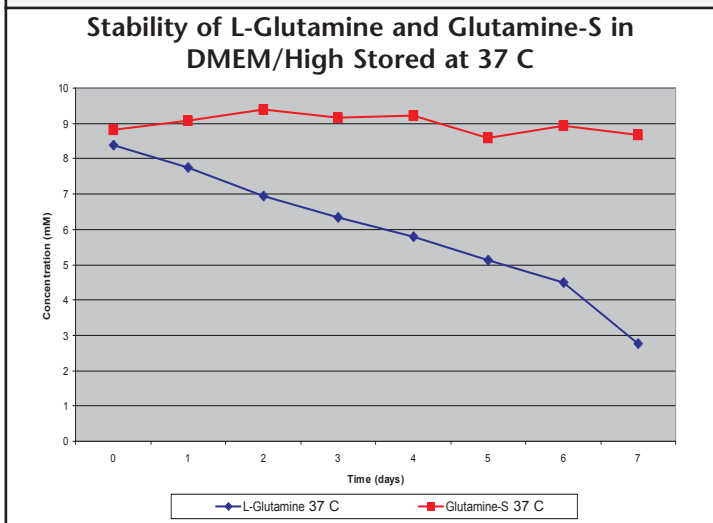
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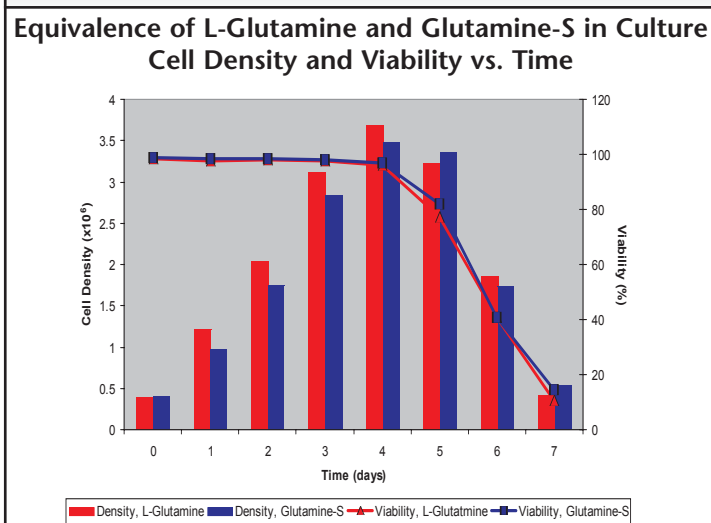
Figure 3



Both L-glutamine and Glutamine-S in medium resisted degradation at 2 to 8 C in DMEM/High for seven days (Figure 5). These solutions remained at consistent concentrations and demonstrated greater stability compared to solutions stored under higher temperatures. In an extended evaluation through six weeks, L-glutamine and Glutamine-S in medium remained within 2 mM of their original concentrations.

In an equivalence evaluation, Chinese Hamster Ovary (CHO) cells supplemented with Glutamine-S were evaluated in comparison to those supplemented with L-glutamine (see Figure 4). CHO cells supplemented with the dipeptide performed similarly to those supplemented with L-glutamine. Further investigations have been conducted which verify equivalent performance, and in some instances enhanced performance, when using the dipeptide supplement. This evidence suggests that similar performance and even enhanced growth and viability could be expected with the use of the Glutamine-S supplement with several mammalian cell line applications.

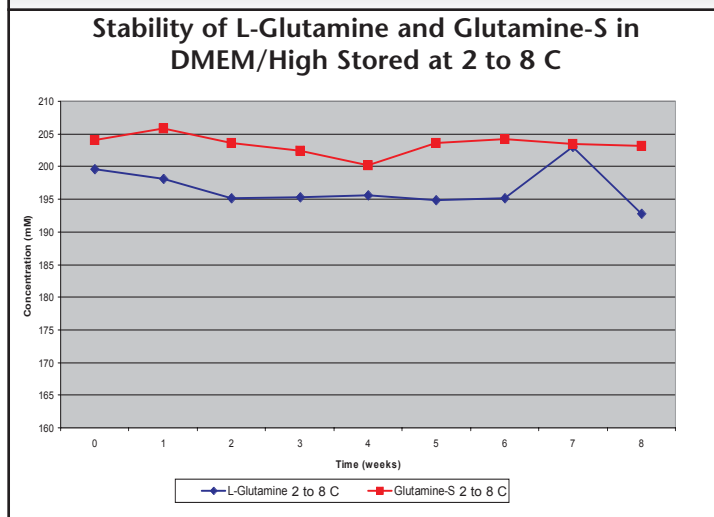
Figure 4



Stability of L-Glutamine Solution 200 mM

In addition to the use of glutamine in working concentrations with cell culture medium, the storage of L-Glutamine Solution 200 mM at 2 to 8 C is of increasing importance when determining the stability of product prior to supplementation. The degradation of 200 mM supplements could cause similar cytotoxicity issues once supplemented into various applications. An investigation of 200 mM stock solutions of L-glutamine and Glutamine-S stored at 2 to 8 C was carried out over eight weeks and analyzed for amino acid concentration (see Figure 5).

Figure 5



Both L-glutamine and Glutamine-S remained stable throughout eight weeks in 200 mM solutions stored at 2 to 8 C. By maintaining high levels of glutamine in 200 mM stock solutions, Glutamine-S further demonstrates how it can serve as an ideal substitution for L-glutamine in cell culture media.

Conclusions

The loss of glutamine concentration in media could compromise cell growth and viability by decreasing the amount of available nutrients to cells and increasing the amount of cytotoxic ammonia present. For storage purposes, cooler temperatures are required to prevent compromising the stability of medium supplemented with L-glutamine. The use of Glutamine-S as a substitute for L-glutamine can serve to increase the stability of supplemented medium, and can produce equivalent performance when used in mammalian cell culture.

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