

## Stability of GlutaMAX™-I vs. L-Glutamine

Glutamine is an essential nutrient in cell cultures for energy production as well as protein and nucleic acid synthesis. A drawback to glutamine in cell culture media is spontaneous breakdown once in solution. Glutamine breakdown generates ammonia as a by-product, which is toxic to the cells (1) and can affect protein glycosylation (2,3). One strategy to minimize generation of ammonia is to replace the L-glutamine in medium with the glutamine dipeptide, GlutaMAX™-I. Figures one and two demonstrate the improved stability of the glutamine dipeptide and reduction of spontaneous ammonia generation.

An additional benefit to using GlutaMAX™-I in place of L-glutamine in cell culture is the extension of culture time, potentially reducing the number of times the cells must be passaged. Figure three is a comparison of MDBK cells cultured in D-MEM with 10% Fetal Bovine Serum (FBS) and either glutamine or GlutaMAX™-I. Cells cultured in GlutaMAX™-I reach peak density two days later and viability declines less rapidly than that observed in cultures with glutamine supplementation.

Many widely used GIBCO® media formulations are available with the GlutaMAX™ dipeptide substituted for L-glutamine. Complete listing of GlutaMAX™ products available. Additionally, the GlutaMAX™ dipeptide is available as a 200 mM solution and can be substituted equally for glutamine on a molar basis. It can be used with most cell lines with equivalent performance and substituted in both serum-supplemented and serum-free formulations requiring L-glutamine.

The slight increase of the lag phase is attributed to the time needed to release the peptidase and digest the dipeptide. This allows a gradual increase in availability of L-glutamine to the cells (1).

### References:

- Hassell, T., Gleave, S., Butler, M. Growth Inhibition in Cell Culture. *Applied Biochemistry and Biotechnology*, Vol. 30, 1991, pp 30-41.
- Yang, M.; Butler, M. Effects on Ammonia and Glucosamine on the Heterogeneity of Erythropoietin Glycoforms. *Biotechnology Progress*, 2002, Vol. 18, 129-138.
- Yang, M.; Butler, M. Effect of Ammonia on the Glycosylation of Human Recombinant Erythropoietin in Culture. *Biotechnology Progress*, 2000, Vol. 16, 751-759.

### Common Cell Lines Cultured with GlutaMAX™-I

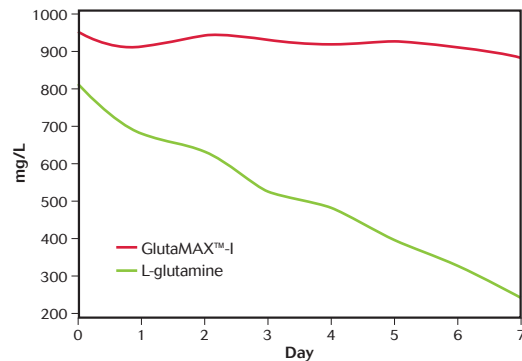
MDBK	Bovine Kidney
MDCK	Canine Kidney
HELA	Human Ovary
Per. C6	Human Embryonic Retinoblastoma
293	Human Embryonic Kidney
AE-1	Mouse Hybridoma
3D9	Mouse Hybridoma
CHO	Hamster Ovary
BHK	Hamster Kidney

See Chapter 1 for ordering information.

### Important Licensing Information

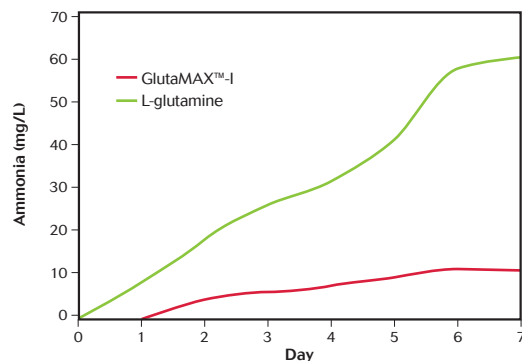
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### Stability of GlutaMAX™-I vs. L-Glutamine in D-MEM at 37°C



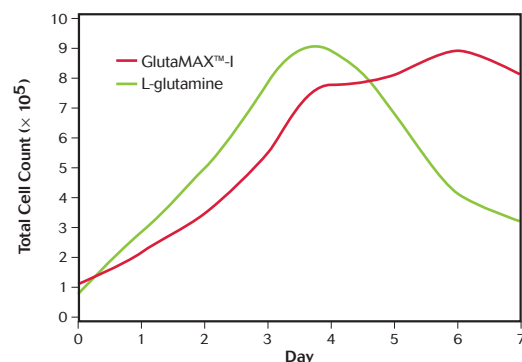
**FIGURE 1** — D-MEM was supplemented with GlutaMAX™-I or L-glutamine, aliquoted into vials and stored at 37°C. Samples were taken daily and frozen at -20°C. Levels of GlutaMAX™-I and L-glutamine were determined by HPLC.

### Ammonia Levels in Supplemented Media Stored at 37°C



**FIGURE 2** — D-MEM was supplemented with GlutaMAX™-I or L-glutamine, aliquoted into vials and stored at 37°C. Samples were taken daily and frozen at -20°C. Levels of ammonia were determined by HPLC.

### Growth of MDBK Cells in D-MEM supplemented with L-glutamine or GlutaMAX™-I and 10% FBS



**FIGURE 3** — MDBK cells were seeded at approximately  $1 \times 10^5$  cells/flask in D-MEM with 10% FBS and L-glutamine or GlutaMAX™-I in 25 cm<sup>2</sup> T-flasks.