LIST BIOLOGICAL LABORATORIES, INC.

540 DIVISION STREET ▪ CAMPBELL ▪ CALIFORNIA 95008-6906 ▪ USA
408-866-6363 ▪ 800-726-3213 ▪ FAX 408-866-6364 ▪ EMAIL info@listlabs.com
WEBSITE www.listlabs.com

Tips for Working with Adenylate Cyclase

In working with adenylate cyclase (ACT, CyaA, AC-Hly), it is important to keep in mind the tendency of this enzyme to rapidly aggregate and lose the cell-invasive activity in solutions that do not contain high concentrations of chaotropic agents. It is a hydrophobic protein and even at protein concentrations as low as 0.05 mg/ml, tends to make biologically inactive oligomers in buffers not containing denaturing concentrations of chaotropic agents. Oligomers still exhibit full ACT enzyme activity, but may not be able to deliver ACT into cells and raise cellular cAMP. This toxin is produced denatured and stabilized in 8 M urea solutions. For maximal biological activity, it is important to store ACT at 100 times the working concentration in 8 M urea, and dilute it out into urea-free buffer just prior to addition to cell suspensions. Removing urea by conventional dialysis is undesirable as it reduces specific toxin activity of ACT approximately 100-fold.

Because even a transient decrease in urea concentration may cause irreversible loss of CyaA activity due to aggregation of the denatured protein, care must be taken when thawing the toxin solutions.

Thawing and Storing

Aliquots sized for single use may be frozen at -20^{o}C to avoid repeated thawing and freezing of samples. Frozen ACT is stable for years, and upon renaturation, if sufficiently diluted-out from urea, it recovers efficiently the activity of binding and penetrating cells. Thaw ACT solutions by hand-swirling the tube, or gently agitate it on a shaker at room temperature. Do not thaw samples on ice because urea precipitation will likely occur, allowing aggregation.

Cell Culture and Techniques for Dilution

For the best activity, ACT needs at least 0.5 mM free calcium ions in cell culture buffer. D-MEM (1.9 mM calcium) is preferred to phosphate-buffered RPMI which chelates calcium.

Avoid serial dilutions of ACT in urea-free buffers, starting from a concentrated stock. The best way to dilute ACT is to pre-dilute it in buffer with 8 M urea to a concentration 100-fold greater than the final needed working concentrations. Pre-dilutions are rapidly diluted-out into the urea-free buffers, if necessary on ice, and are quickly added to cells to avoid loss of activity due to aggregation. Use of an inert carrier protein in urea-free dilution buffers, such as 0.1% BSA is highly recommended for diluting toxin. With this method of pre-dilution into a 100-fold toxin concentration in 8 M urea, cultured cells are provided with a consistent, non-toxic concentration of 80 mM urea. Maintaining toxin in 8 M urea, where it is highly stable and making final dilutions, produces consistent results.