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Product #188L
Lot #1888A2
Release Date: April 2017
Recommended Retest Date: April 2020

CERTIFICATE OF ANALYSIS
ADENYLATE CYCLASE TOXIN, RECOMBINANT
Lot #1888A2

Contents

Each vial contains 50 µg of Adenylate Cyclase Toxin (ACT, CyaA, AC-Hly), recombinant (rACT) in 50µL of 0.05 M Tris, 8 M Urea, 0.002 M EDTA, pH 8.0. Gently mix to obtain a uniform solution. **Carefully read the Packaging/Storage/Thawing section below before using this product. Handle the product gently; do not vortex.**

Concentration

The concentration of this product is determined by absorbance at 280 nm.

Purity

When examined on 3-8% SDS-PAGE gels, this protein migrates as a single major band with an apparent molecular weight of approximately 200,000 Da. Densitometric analysis estimates the purity of the product as 80%.

The endotoxin content, determined using a kinetic chromogenic LAL assay, is <150 EU/mg.

Toxicity

The enzymatic activity of this preparation is approximately 780 µmoles cAMP/min/mg of protein as determined by a modification of Hewlett et al.[1]

Cytotoxicity testing on J774 cells indicates an EC_{50} (half maximal effective concentration) of approximately 3.4 µg/ml.

Since each cell type exhibits a different sensitivity, testing a range of toxin concentration is highly recommended.

Impact of Urea

ACT is produced denatured and stabilized in 8M urea solutions. In working with ACT, it is important to keep in mind the tendency of ACT to rapidly aggregate in solutions that do not contain high concentrations of chaotropic agents such as urea, and, subsequently lose cell-invasive activity. ACT is a hydrophobic protein and even at concentrations as low as 0.05mg/ml, tends to form biologically inactive oligomers in buffers not containing denaturing concentrations of chaotrpoic agents. Oligomers still exhibit full AC enzyme activity, but may not be able to deliver AC into cells and raise cellular cAMP.

(Continued)

Techniques for Dilution

For best results, maintain toxin in 8 M urea, where it is highly stable, and work rapidly to make the final dilutions. Pre-dilute the ACT in buffer with 8 M urea to a concentration 100 fold greater than the final working concentration. At the point of use, rapidly dilute the stock into urea-free buffer, if necessary on ice, and add to cells rapidly.

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

Use of a companion protein in urea-free dilution buffers, such as 0.1% BSA is highly recommended for diluting toxin. Removing urea by conventional dialysis is not recommended as it reduces specific toxin activity of ACT approximately 100-fold.

Packaging/Storage/Thawing

This product is supplied as an aseptically packaged liquid. Aliquots, sized for single use, may be frozen at -20°C to avoid repeated thawing and freezing of samples. Frozen ACT is stable for years, and upon renaturation, if sufficiently diluted out of urea, it efficiently recovers the activity of binding and penetrating cells.

Because even a transient decrease of urea concentration may cause irreversible loss of ACT activity due to aggregation of the denatured protein, care must be taken when thawing the toxin solutions. Thaw the ACT solutions by swirling the tube in your hand, or having it gently agitated on a shaker at ambient room temperature. Do not thaw samples on ice because urea precipitation will likely occur, allowing aggregation.

After thawing, to ensure recovery of vial contents, centrifuge before opening the tube. Aseptic handling is recommended, no preservatives have been added to the product.

Handling

Good laboratory technique should be employed in the safe handling of this product. Wear appropriate laboratory attire including lab coat, gloves and safety glasses. Nitrile gloves are recommended when handling lyophilized material.

This product is intended for research purposes by qualified personnel. It is not intended for use in humans or as a diagnostic agent. List Biological Laboratories, Inc. is not liable for any damages resulting from the misuse or handling of this product.

FOR RESEARCH PURPOSES ONLY. NOT FOR HUMAN USE.

References

1. Hewlett, E.L., Gordon, V.M., McCaffery, J.D., Sutherland, W.M. and Gray, M.C. (1989) *J. Biol. Chem.* **264**, 19379-19384.

Production: SH Date: 4/25/17 Management: KD Date: 4-25-17 QA/QC: CLP Date: 4/25/17

Made in U.S.A.