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Certificate of Testing

Adenylate Cyclase Toxin, Recombinant

Prod. No: 188L Lot Number: 18810A2

Date of Manufacture 11 April 2023 **FOR RESEARCH PURPOSES ONLY. NOT FOR HUMAN USE.**

Contents

Each container holds 50 µg of Adenylate Cyclase Toxin (ACT, CyaA, AC-Hly), recombinant (rACT), in 39 µL of 0.05 M Tris-HCl, 8 M Urea, pH 8.0. Concentration is 1.3 mg/mL. Gently mix to obtain a uniform solution. **Carefully read the Thawing section below before using this product. Handle the product gently; do not vortex.**

Storage/Shipping Requirements

Store at -20°C. This product is provided as an aseptically packaged frozen liquid. Ship frozen on dry ice.

Handling

Good laboratory technique should be employed in the safe handling of this product. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses.

The 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.

Attribute	Test Method ID/Version	Results
Appearance	Physical Appearance / ASSAY.063 v05	Clear, colorless, particulate free
Purity	OD Measurement / ASSAY.060 v07	A ₂₈₀ /A ₂₆₀ : 1.5 A ₃₂₀ : 0.002
	SDS-PAGE / GELS.018 v18	Non-Reduced: 94% purity Reduced: 94% purity
	Endotoxin / ASSAY.092 v06	2.4 EU/mg
Activity	J774A.1 Cell Assay / ASSAY.168 v01	Cytotoxicity CC ₅₀ : 10 ng/mL
	Enzyme Specific Activity ¹	767 µM cAMP/min/mg toxin

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



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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.

Techniques for Dilution

When stored in an 8 M urea solution (where it is highly stable), the toxin is denatured. If sufficiently diluted out of urea, the toxin efficiently recovers the activity of binding and penetrating cells. To renature the toxin and prepare it for cell testing in a urea-free buffer, for best results, remove the frozen toxin from storage and initially maintain it in the 8 M urea solution. Next, work rapidly to make the final dilutions.

First, pre-dilute the ACT in buffer with 8 M urea to a concentration 100 fold greater than the final working concentration. Next, at the point of use, rapidly dilute the stock toxin 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.

Thawing

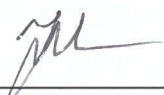

Aliquots, sized for single use, may be frozen at -20°C to avoid repeated thawing and freezing of samples.

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.

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.

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Production:  Date: 05 May 2023