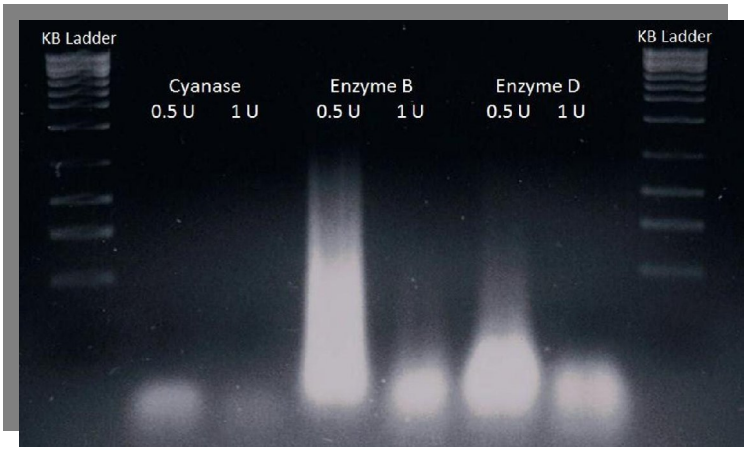
Cyanase™ Nuclease System

Effective For:

- Cell Lysate Clearance
- Protein and Viral Purification
- Nucleic Acid Removal from Samples
- Degrades all forms of DNA and RNA

Unique Features:

- Easily Inactivated and Removed
- Faster than any Current Nuclease on the Market
- No measurable loss of activity up to 1 year at room temperature



Concentration: 50 U/µl

Purity: No contaminating bands detected by loading 5 ug of Cyanase on a 4-20% Tris-Glycine SDS gel and staining with SimplyBlue™ (Invitrogen Corporation) gel stain for 1 hour.

Unit Definition: One unit is the amount of enzyme that degrades 3 µg of Lambda DNA completely at 37ºC in 1 minute. Reaction conditions are 50 mM Tris pH 8.0, 6 mM $MnSO_4$.

Storage Buffer: 50 mM Tris pH 8.0, 5 mM $MgSO_4$, 50% Glycerol. Store at -20ºC.

Figure1 –Superior Activity Compared to other Commercial Nucleases.
3 µg of full length λ DNA was incubated at 37°C for 1 minute with various nucleases in their optimal reaction buffers. **Lane 1,8:** 1.0 kb marker **Lane 2:** 0.5 U Cyanase **Lane 3:** 1 U Cyanase **Lane 4:** 0.5 U Enzyme B from *Serratia marcescens* **Lane 5:** 1 U Enzyme B from *Serratia marcescens* **Lane 6:** 0.5 U enzyme D from bovine **Lane 7:** 1 U enzyme D from bovine.

The new Benzonase® alternative, Cyanase is a cloned highly active non-Serratia based non- specific endonuclease that degrades single and double stranded DNA and RNA in as little as 1 minute. The Cyanase system is unique in its ability to be easily removed from samples after the reaction is finished using the novel Cyanase inactivation resin. The resin can be easily filtered or spun down to remove from the sample removing the Cyanase with it. Cyanase is active over several conditions as outlined in Table 1 and Figure 2. Cyanase is fully active in DTT up to 100 mM, and most common non-ionic detergents up to 1% including Triton X-100, Tween 20, and Igepal CA630 .Cyanase is active in Urea up to 3 M.

Cyanase is fully active in all common chemical lysate treatments such as lysozyme or detergent. In lab studies, Cyanase has shown minimal loss of activity as measured by lysate clearance assay after 1 year at room temperature. No other competitor comes close. Cyanase is completely inhibited by phosphate buffers at 20 mM or higher, and NaCl concentrations of 200 mM or greater. For optimal activity, it is highly recommended to use Mn^{2+} in place of Mg^{2+} and avoid any chelating reagents as the enzyme requires divalent cations for activity. For pH use greater than 8.3 or less than 7.0, Mg is recommended for maximum activity due to Mn instability.

Table 1 Common Conditions for Cyanase Activity

Conditions	Optimal	Active
pH	8.0	6.0-10.0
Temperature	37ºC	0-50ºC
NaCl	0 mM	0-100 mM
Mn^{2+}	6-10 mM	1-10 mM
Mg^{2+}	10 mM	1-10 mM

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WIDE RANGE OF APPLICATIONS

NO MORE WORRIES ABOUT PURIFICATION METHODS TO REMOVE THE NUCLEASE

OEM AND BULK OPPORTUNTIES WELCOME

LET US PARTNER WITH YOUR BUSINESS

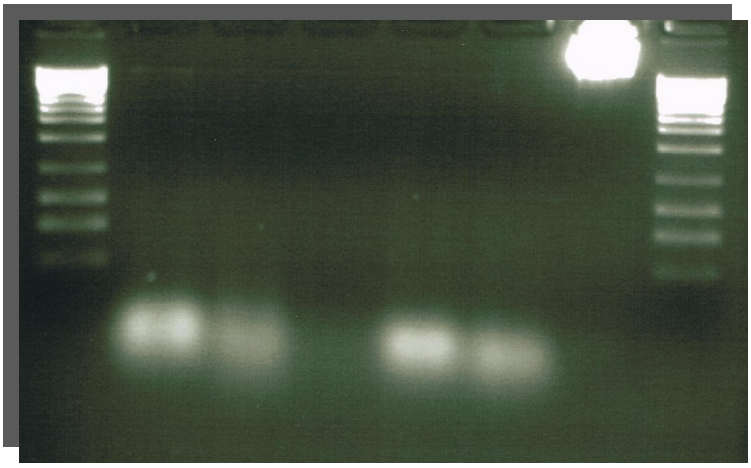

Figure 2: Fast Digestion of DNA over a Wide Range of Conditions
1 U of Cyanase was incubated with 3 µg of Lambda DNA for 3 minutes at 37ºC at various conditions. **Lane 1:** 1.0 kb marker **Lane2:** 1 U Cyanase pH 6.0 10 mM $MgSO_4$. **Lane3:** 1 U Cyanase pH 7.0, 6 mM $MnSO_4$. **Lane 4:** 1 U Cyanase pH 8.0, 6 mM $MnSO_4$. **Lane 5:** 1 U Cyanase pH 8.5, 10 mM $MgSO_4$. **Lane 6:** 1 U Cyanase pH 10.0, 10 mM $MgSO_4$. **Lane 7:** 3 µg undigested lambda DNA. **Lane 8:** 1.0 kb marker.

Application Guidelines

For Cell Lysate Clearance: 10-50 U of Cyanase can be added to the lysate buffer per gram of cell paste. A typical lysate buffer would be 50 mM Tris pH 7.5-8.0, 0-20 mM NaCl, and 1 mg/ml lysozyme. 6 mM $MnSO_4$ is usually added fresh from concentrated frozen stock provided with the Cyanase. The Cyanase is incubated with the cell paste for up to 20-30 minutes at 0-37ºC or until the cells are fully lysed and non-viscous. Cyanase will have maximum activity at room temperature or higher although acceptable activity is observed down to 0°C. Optimal activity is achieved when incubated with the cell paste during lysis and keeping at least 10 U of enzyme per gram of cell paste. 50 U of Cyanase/g of cell paste will clarify the solution in as little as 2 minutes or less in optimal conditions even in highly viscous reactions. The cells can then be spun down after lysis and are ready to proceed to downstream capture. Alternately, Cyanase™ Inhibitor resin (Cat#1010, 1020) can be added to the cell lysate after incubation and spun as before to remove the Cyanase from the reaction.

For Nucleic Acid Removal in Protein Samples: 10 - 50 U of Cyanase per sample can be added depending on DNA or RNA levels and incubate at 30-37ºC for 10 minutes in optimal conditions. Removal of the Cyanase can be achieved through addition of 100 µl of Cyanase™ Inactivation Resin per ml of sample.

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Cyanase™ Inactivation Resin

EASY TO USE

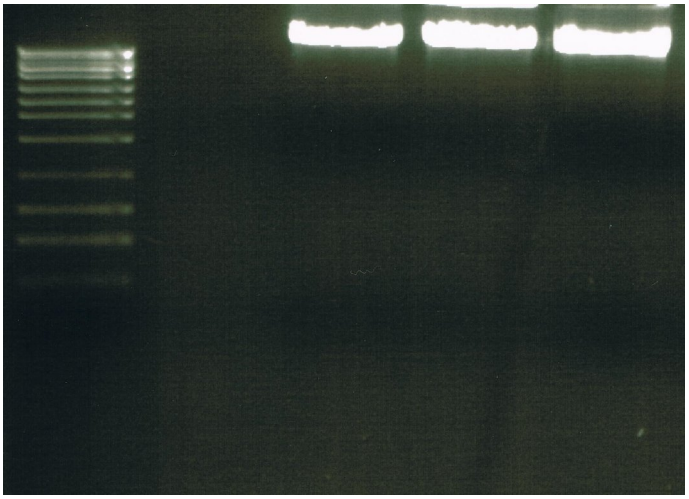
NO OPTIMIZATION NEEDED

ACTIVE OVER A WIDE RANGE OF CONDITIONS

NO INTERACTIONS WITH OTHER PROTEINS OR TARGETS

EXTREMELY TIGHT INTERACTION BETWEEN CYANASE AND RESIN.

GREATER THAN 99.9% REMOVAL IN AS LITTLE AS 20 MINUTES FOLLOWING OUTLINED PROCEDURES



Average Particle Size: 90 µm in 50% buffer slurry.

Product Information: Inactivation resin is fully active under all normal Cyanase reaction conditions. In addition inactivation resin has been tested down to pH 4.8, up to pH 10.0, down to 0ºC, and in 500 mM NaCl with no effect on Cyanase removal. Inactivation resin has no affinity for any other proteins or reagents.

Figure 3: Complete Removal of Nuclease Activity
Various amounts of Cyanase were incubated with inactivation resin at room temperature and spun down to remove the resin. The resulting supernatants were incubated with 1 µg λ DNA for 1 hour at room temperature. **Lane 1:** 1.0 kb marker, **Lane 2:** 10 µl untreated 50 U/ml Cyanase sample incubated for 1 minute with λ DNA at RT.. **Lane 3:** 10 µl 50 U/100 µl Cyanase sample treated with 50 µl 50% resin slurry for 20 minutes at RT. **Lane 4:** 10 µl of 50 U/100 µl Cyanase sample treated with 25 µl 50% resin slurry for 1 hour at RT. **Lane 5:** 20 µl of 100 U/ml Cyanase sample treated with 100 µl 50% resin slurry. After 1 hour with the aliquots, no activity is detected versus the untreated lane degrading the entire sample in less than 1 minute.

Cyanase Inactivation resin is a proprietary blend of Cyanase inhibitor protein coupled to Sepharose Fast Flow resin creating a novel method for inactivating and removing the Cyanase enzyme prior to downstream applications. No more worries about downstream contamination with nucleases or difficult removal processes which may compromise product integrity. Cyanase Inactivation resin makes the removal simple and easy to follow in as little as 20 minutes. To remove Cyanase, simply add 10 µl of 50% slurry for every 10 U if Cyanase or for every 100 µl of sample, whichever is greater. The sample can then be inverted, pipetted or stirred for 20 minutes to 1 hour at room temperature to provide continuous mixing. The sample is then spun down at 4-5,000 Xg for 5 minutes and the soluble sample removed.

Alternatively, less resin can be used if incubated for longer periods of time. As an example, 5 µl of 50% resin can be added for every 10 U of Cyanase if incubated with continuous mixing for 1 hour to overnight. It is recommended that 10 µl of resin always be used for every 100 µl of reaction regardless of Cyanase concentration for optimal effectiveness regardless of incubation time. For samples requiring more than 1 ml of resin slurry, the resin can be packed into a column and the sample passed over the resin at 15 cm/hour or less. The capacity of a packed bed will be on average 10X greater than using the slurry method, and the sample can be passed over the resin multiple times to remove trace contamination. There is no interaction between the resin and target proteins.

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Ordering Information

Sample Size	Catalog Number	Standard Price
10,000 U Cyanase, 1.0 mL of 1 M $MnSO_4$	1000	$80.00
25,000 U Cyanase, 1.0 mL of 1 M $MnSO_4$	1005	$165.00
100,000 U Cyanase, 1.0 mL of 1 M $MnSO_4$	1030	$465.00
Nucleic Acid Removal Kit (10,000 U Cyanase, 1 mL Inactivation Resin, and 1 mL of 1 M $MnSO_4$)	1050	$125.00
1 mL Cyanase Inactivation Resin (50% slurry, 0.5 mL beads)	1010	$50.00
5 mL Cyanase Inactivation Resin (50% slurry, 2.5 mL beads)	1020	$225.00

RiboSolutions is dedicated to bringing novel nuclease and enzyme based products to the life science industry. We're not just a reagents company. We believe in building partnerships with biotechnology researchers and companies. Let us build one with your company today. Visit us at www.ribosolutionsinc.com to order.