Binuclease, Lyophilized

Non-specific Endonuclease — lyophilized powder

Cat. No. EV-NUC-005 | Version 1.2 | May 2026

Cat. No. EV-NUC-005 Size 100 kU per tube (≥20 kU/mg powder) Storage ≤4°C

1. Overview

Binuclease (EV-NUC-005) is a genetically engineered non-specific endonuclease derived from Serratia marcescens and expressed in a yeast platform. The product is supplied as a sterile lyophilized powder with activity ≥20 kU per mg of dry powder and specific activity ≥1000 kU per mg of protein. Purity is ≥90% by SDS-PAGE (Grade I). The enzyme digests all forms of DNA and RNA — single-stranded, double-stranded, linear, circular, and supercoiled — in a non-sequence-dependent manner, cleaving phosphodiester bonds to produce 5'-monophosphate-terminated oligonucleotides 2–5 bases in length. Active across pH 6–10 (optimum 8.0) and 0–42°C (optimum 37°C) in the presence of 1–10 mM Mg^{2+} cofactor. Used for nucleic acid removal in vaccines, monoclonal antibodies, recombinant proteins, viral vectors, and cell therapy products, and for viscosity reduction in cell lysates during downstream bioprocessing.

2. Mechanism of Action

Binuclease hydrolyzes internal phosphodiester bonds of nucleic acid substrates without regard to sequence or secondary structure. The enzyme requires divalent magnesium ions (Mg^{2+}) as a catalytic cofactor at 1–10 mM working concentration (optimum 2 mM). Cleavage proceeds processively until substrates are reduced to 5'-monophosphate oligonucleotides 2–5 nucleotides in length, which are too short to support secondary structure, hybridization, or template activity. The enzyme is fully active across pH 6.0–10.0 (optimum pH 8.0) and 0–42°C (optimum 37°C). Activity is inhibited by EDTA chelation of the magnesium cofactor and by elevated monovalent salt concentrations. Heat denaturation at 70°C for 30 minutes provides complete inactivation.

3. Applications

- Removal of host-cell DNA and RNA from recombinant protein and monoclonal antibody preparations
- Nucleic acid clearance in viral vector, vaccine, and cell therapy downstream processing
- Viscosity reduction in cell lysates to facilitate filtration, centrifugation, and chromatography
- Sample preparation for 2D gel electrophoresis, ELISA, and proteomics workflows
- AAV vector and inclusion body purification — improved yield and purity of cell-derived particles
- Footprint analysis and protein mapping with improved resolution and sample recovery

4. Recommended Protocol

Step	Details
1. Equilibrate lyophilized Binuclease tube to room temperature for 5 minutes, then briefly centrifuge at 1,000 × g	Prevent condensation

Step	Details
for 5 seconds to collect material at the bottom.	
2. Reconstitute with sterile nuclease-free water to the desired working concentration. For a 250 U/µL stock, add 400 µL ultrapure water to a 100 kU tube. Adjust volume per lot-specific activity printed on the Certificate of Analysis.	250 U/µL stock
3. Close the tube tightly and invert 10 times to dissolve. Do not vortex vigorously. Allow the solution to sit at room temperature for 2–3 minutes, then mix again by inversion. Solution should appear clear to slightly opalescent.	Gentle mixing
4. Aliquot reconstituted enzyme into nuclease-free tubes (50–200 µL per tube). Store aliquots at -20°C for up to 6 months; reconstituted enzyme is stable at 4°C for up to 1 week for immediate use.	Aliquot and store
5. Add $MgCl_2$ to the sample to a final concentration of 1–10 mM (optimum 2 mM). Verify sample pH is 6.0–10.0; adjust to 8.0 with Tris-HCl for maximum activity.	Cofactor + pH
6. Add reconstituted Binuclease to the sample at 1–2.5 U per mL of sample for moderate nucleic acid load; 10–25 U/mL for heavily contaminated or viscous lysates. Mix gently.	Standard working conc.
7. Incubate at 37°C for 30 minutes; extend to 60 minutes for viscous lysates. Mix gently every 10 minutes.	Digestion
8. Verify digestion by observed viscosity reduction. Optionally measure A_{260} before and after; a reduction of >95% indicates successful digestion. Inactivate by heat (70°C, 30 min) or EDTA (10 mM final) if downstream steps require.	Verification + stop

Working concentration ranges: DNA/RNA clearance in recombinant protein and antibody preparations 1–2.5 U/mL; viscosity reduction in viscous lysates 10–25 U/mL; viral vector and vaccine bioprocessing per process-specific qualification.

5. Unit Definition / Activity Specification

One unit (U) of Binuclease digests sonicated salmon sperm DNA to produce acid-soluble oligonucleotides equivalent to a ΔA_{260} of 1.0 in 30 minutes at pH 8.0 and 37°C (Sigma-Aldrich method). The product is supplied at ≥20 kU per mg of dry lyophilized powder. Specific activity is ≥1000 kU per mg protein.

6. Quality Control

Test / Parameter	Specification	Lot Result	Status
Appearance	White lyophilized powder	Conforms	**PASS**
Activity per Powder	≥20 kU/mg dry weight	≥22 kU/mg	**PASS**

Test / Parameter	Specification	Lot Result	Status
Specific Activity	≥1000 kU/mg protein	Conforms	**PASS**
Purity (SDS-PAGE)	≥90% (Grade I)	Conforms	**PASS**
Protease Contamination	No detectable activity	None detected	**PASS**
Endotoxin	Below detection limit (endotoxin assay)	None detected	**PASS**
Functional Verification	Complete degradation of dsDNA and total RNA to 2–5 nt oligonucleotides under standard conditions	Conforms	**PASS**

7. Storage & Stability

- Storage temperature: ≤4°C for lyophilized powder
- Stability: 3 years at ≤4°C (lyophilized); 6 months at -20°C (reconstituted aliquots); 1 week at 4°C (reconstituted, for immediate use)
- Formulation: sterile lyophilized powder
- Shipping: room temperature acceptable (lyophilized form is shelf-stable)
- General: avoid repeated freeze-thaw of reconstituted enzyme; aliquot upon reconstitution; working solution stable on ice for up to 8 hours; maximum 3 freeze-thaw cycles recommended

8. Troubleshooting

Problem	Possible Cause	Suggested Action
No or low nucleic acid degradation	Missing or insufficient Mg^{2+} cofactor; EDTA in sample chelating Mg^{2+}; high monovalent salt (>150 mM NaCl)	Verify $MgCl_2$ at 2 mM final; if EDTA is present, add compensating $MgCl_2$ to exceed EDTA molar concentration by 2 mM; dilute sample to reduce salt
Persistent sample viscosity after standard digestion	Heavily contaminated lysate; insufficient enzyme dose or time	Increase enzyme to 10–25 U/mL; extend incubation to 60 minutes; ensure 37°C throughout
Loss of activity in stored aliquots	Repeated freeze-thaw; extended storage at 4°C	Aliquot upon reconstitution; limit to 3 freeze-thaw cycles; use 4°C-stored reconstituted enzyme within 1 week
Cloudy reconstituted solution	Vigorous vortexing causing protein denaturation; out-of-	Reconstitute by inversion only; verify reconstitution buffer pH is

Problem	Possible Cause	Suggested Action
	range pH	6–10; centrifuge briefly at 10,000 × g and use supernatant
Residual enzyme activity in downstream sample	Inactivation step omitted or insufficient	Inactivate by heating at 70°C for 30 minutes, or add EDTA to 10 mM final to chelate Mg^{2+} cofactor

9. Safety Information

For Research Use Only. Not for diagnostic or therapeutic use. Handle according to standard laboratory safety guidelines. Wear laboratory coat, protective gloves, and safety eyewear when handling this product. Refer to the accompanying Safety Data Sheet (SDS) for full hazard information. Dispose in accordance with local, state, and federal regulations.