GENERAL DESCRIPTION

J.T.Baker® Endonuclease is designed for the degradation of both single stranded and double stranded DNA and RNA and is available in a variety of sizes and grades to support processes from development to large scale commercial batches. J.T.Baker® Endonuclease Ultrapure Bioreagent is suitable for use in research and development environments and is offered in three units of measure; 100 KU, 1,250 KU, and 5,000 KU. J.T.Baker® Endonuclease Biotech Reagent is manufactured under cGMP, for use in processes requiring greater regulatory control and is available in units of measure from 100 KU to 25,000 KU. Please refer to JTB.00020.03, J.T.Baker® Endonuclease Biotech Reagent Technical Data Sheet, for additional information. Chemical definitions and grades may be found at Resource library | Avantor (avantorsciences.com).

J.T.BAKER® ENDONUCLEASE FREQUENTLY ASKED QUESTIONS

QUESTION 1: What is the application of J.T.Baker® Endonuclease?

J.T.Baker® Endonuclease is an enzyme derived from the native endonuclease of Serratia marcescens. Endonuclease cleaves both single stranded and double stranded DNA and RNA. J.T.Baker® Endonuclease can be used during manufacture of recombinant proteins and gene therapies, such as AAV manufacturing, to cleave residual plasmid DNA used in transfection and host cell DNA/RNA introduced into the medium during cell lysis. By degrading these impurities, J.T.Baker® Endonuclease helps reduce viscosity, which can interfere with downstream processing steps. Additionally, J.T.Baker® Endonuclease may be used at the end of fermentation to reduce viscosity caused by DNA and RNA impurities in the medium due to cell autolysis. The increased viscosity may impact critical parameters such as gas and nutrient exchange rates, making it challenging to maintain control of the bioreactor.

QUESTION 2: What is the composition of J.T.Baker® Endonuclease?

J.T.Baker® Endonuclease is a recombinant enzyme produced in bacterial cell line (*E. coli)* and subsequently purified. The purified enzyme is formulated using 20 mM Tromethamine (TRIS), 2 mM Magnesium Chloride Hexahydrate, 20 mM Sodium Chloride, 50% (v/v) Glycerin, and >250 U/µL endonuclease. After formulation, the product is 0.2 µm filtered to provide a final product that is consistent with product specifications suitable for use in biopharmaceutical manufacturing applications. J.T.Baker® Endonuclease Ultrapure Bioreagent and J.T.Baker® Endonuclease Biotech Reagent have the same composition.

QUESTION 3: What is the definition of a unit of J.T.Baker® Endonuclease?

A unit of J.T.Baker® Endonuclease is the amount that can degrade a sample of DNA (sonicated salmon sperm DNA), producing acid-soluble oligonucleotides, to cause a specific, measurable change in light adsorption equivalent to ΔA_{260} of 1.0 in 30 minutes.

QUESTION 4: What is the mechanism of J.T.Baker® Endonuclease?

J.T.Baker® Endonuclease cleaves both single stranded and double stranded DNA and RNA enabling rapid clearance of residual DNA and RNA.[1] (Meiss, 1995) In AAV vector bioprocessing, J.T. Baker® Endonuclease has been shown to retain enzymatic activity in the presence of either J.T. Baker® Cell Lysis Solution, (product code JTG193) or Polysorbate 20, enabling robust and efficient cell lysis and DNA/RNA degradation in a single step.

QUESTION 5: What is the recommended usage or working concentration of J.T.Baker® Endonuclease?

J.T.Baker® Endonuclease can be added to lysed cellular contents at a target concentration of 25 – 100 U/ml in combination with 2 – 5 mM Magnesium Chloride Hexahydrate, part number JT2449, at 37°C and pH range of 8.0 – 9.0. Please note that we recommend optimization studies to find the ideal conditions for your process and product. Please refer to JTB.00020.03, J.T.Baker® Endonuclease Biotech Reagent Technical Data Sheet, instructions for use for additional information.

QUESTION 6: Can J.T.Baker® Endonuclease be removed from the intermediates during downstream processing?

Yes. J.T.Baker® Endonuclease enzyme can be removed during affinity chromatography step as it does not bind to AAV-specific affinity ligands and will be washed away in flow-through or wash fractions. Additionally, the ultrafiltration/tangential flow filtration process can effectively remove the enzyme since AAV processes typically use membranes with a molecular weight cut-off (MWCO) greater than 100kDa, while the size of the endonuclease is less than 30kDa.

QUESTION 7: Does Avantor have an assay for detection of J.T.Baker® Endonuclease?

Yes. A critical aspect of this process is the detection of residual endonuclease in the downstream product. If present in the final product, it can potentially degrade therapeutic genetic material, leading to reduced potency or unwanted immune responses in patients. J.T.Baker® Endonuclease can be quantified using any commercially available endonuclease residual detection ELISA kit such as CYGNUS®2 EndonucleaseGTP®-F960 ELISA Kit, ELISA KIT ENDONUCLEASE GTP | VWR or EndonucleaseGTP ELISA Cygnus F960 at Our Products & Analytical Methods | Cygnus Technologies outside of the US. Please refer to Technical Note JTB.00006.00, J.T.Baker® Endonuclease Detection using Enzyme linked immunosorbent Assay (ELISA) that confirms the reliability of commercially available ELISA kits to quantitatively determine residual levels of J.T.Baker® Endonuclease.

QUESTION 8: Does J.T.Baker® Endonuclease contain any components of animal origin?

No. J.T.Baker® Endonuclease is recombinant enzyme produced in bacterial cell line (*E.Coli*) and subsequently purified. J.T.Baker® Endonuclease is animal origin free (AOF)/non animal derived (non-ADR) – manufactured from raw materials that contain NO animal parts, products, and/or by-products. The components of the formulation buffer are chemicals of non-animal origin. Both Ultrapure Bioreagent (BAKR7108) and Biotech Reagent (BAKRGT20) are animal origin free, having the same process and formulation from non-GMP to GMP, allowing process developers to scale with confidence. Avantor provides corresponding Regulatory Support File (RSF), including BSE/TSE safety statement. Please request via our representatives.

QUESTION 9: Does J.T.Baker® Endonuclease require special storage and shipping conditions?

Yes. J.T.Baker® Endonuclease is stored at -20°C ±.10°C. While storage at -20°C is recommended, the enzyme's stability was assessed across a range of temperatures from -80°C to 25°C to demonstrate its reliability under different conditions. J.T.Baker® Endonuclease activity remained in specification and within 14% of initial timepoint testing for both ambient and 2-8°C storage temperatures up to 24 weeks. Refer to Lab Report JTB.00005.00, Stability of J.T.Baker® Endonuclease. Additionally, the enzyme activity remained within specifications when stored at -80°C for up to 30 days (see Figure 1). Having the option to store the product in interim temperature conditions offers flexibility and easier handling, ensuring it can be seamlessly integrated into a wide range of manufacturing environments. However, storage at or below -80°C is not recommended for long-term storage.

J.T.Baker® Endonuclease Biotech Reagent part numbers BAKRGT20-50, BAKRGT20-62, and BAKRGT20-80 are shipped in temperature-controlled shippers, monitored using cold chain logistics. This specialized packaging ensures the reagents maintain stable temperature conditions of -20°C ± 5°C. J.T.Baker® Endonuclease Ultrapure Bioreagent and J.T.Baker®

Endonuclease Biotech Reagent less than 5,000 KU are shipped in insulated shippers using icepacks able to maintain -30°C to 30°C for up to five-days. Shipping stability is detailed in Lab Report JTB.00009.00, J.T.Baker® Endonuclease Shipping Stability.

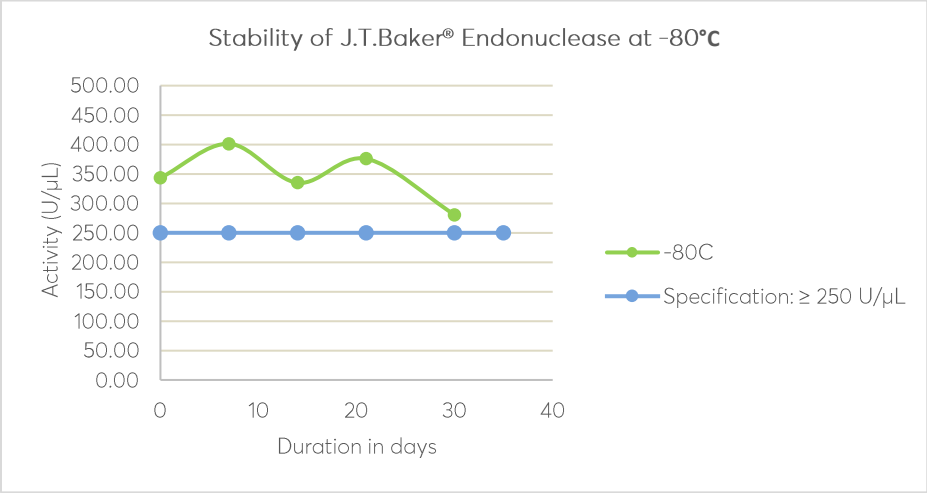

Figure 1: Stability of J.T.Baker® Endonuclease at -80°C

QUESTION 10: What is the shelf life of J.T.Baker® Endonuclease?

J.T.Baker® Endonuclease shelf life if 24-months after the date of manufacture when stored at -20°C ± 10°C, established through GMP stability studies.

QUESTION 11: Can J.T.Baker® Endonuclease cleave my protein of interest?

No. J.T.Baker® Endonuclease is a sugar nonspecific hydrolase that specifically cleaves DNA and RNA. Its enzymatic activity specifically cleaves phosphodiester bonds in nucleic acids. It does not affect peptide bonds between amino acids in proteins, ensuring therapeutic proteins or viral capsid components remain intact. The cGMP-grade product (J.T.Baker® Endonuclease Biotech reagent) is tested for the absence of protease activities to ensure specific application of the product.

QUESTION 12: Does Avantor have comparative DNA/RNA degradation data for similar/competitive products?

Yes. Investigating DNA degradation is crucial for understanding enzyme efficiency. DNA degradation was evaluated using J.T.Baker® Endonuclease and a competing enzyme based upon the native endonuclease of *Serratia marcescens*. Host cell DNA was measured using Human Host Cell DNA kit to detect residual human hcDNA levels. Results indicated that J.T.Baker® Endonuclease degraded hcDNA to concentrations comparable to those achieved by competitive enzyme, demonstrating its robust performance. Experiments were conducted using 100 U/mL endonuclease, 5mM $MgCl_2$, in AAV2, clarified samples, for 2 hours at 37°C while mixing at 125 rpms in presence of cell lysis detergent.

QUESTION 13: Is J.T.Baker® Endonuclease compatible with cell lysis detergents?

Yes. The compatibility of cell lysis detergents/solutions with J.T.Baker® Endonuclease for DNA digestion was evaluated to confirm the enzyme's activity is not inhibited by common detergents such as J.T.Baker® Cell Lysis Solution or Polysorbate 20. J.T.Baker® Cell Lysis Solution, product number BAKRG193, is an effective, ready-to-use solution containing a proprietary blend of readily biodegradable, nonionic surfactants used to solubilize the cell membrane to release AAV from the host cells.

To confirm J.T.Baker® Endonuclease can be used during cell lysis, the enzyme was added to the recommended use dilution, as well as increasing concentrations, of J.T.Baker® Cell Lysis Solution. Relative to the control, J.T.Baker Endonuclease® retained

greater than 95% enzymatic activity and showed DNA clearance in the internal testing. Refer to *Avantor (2025). Novel Cell Lysis Solution: Scaling up viral vector harvest with ease and regulatory compliance [Whitepaper].*

QUESTION 14: What are the product specifications and product options available for J.T.Baker® Endonuclease?

J.T.Baker® Endonuclease available part numbers and specifications provided in Table 1.

Table 1: Product information and specifications

Part Number	Unit of measure	Storage	*Test/Specifications
BAKRGT20-10	100 KU	At or below -20°C	Purity by HPLC — ≥ 99% Appearance — Clear, colorless Identification — Passes Test Activity — ≥ 250 Units/µL Specific Activity — ≥ 1.1 x 10^6 Units/mg Endotoxin — ≤ 0.25 EU/1000 Units Total Aerobic Microbial Count — ≤ 10 CFU/100,000 Units Total Yeast and Mold Count — ≤ 10 CFU/100,000 Units Protease — None detected.
BAKRGT20-25	500 KU		
BAKRGT20-40	1,250 KU		
BAKRGT20-50	5,000 KU		
BAKRGT20-62	12,500 KU		
BAKRGT20-80	25,000 KU		

Table 1 provides information for J.T.Baker® Endonuclease Biotech Reagent, for use in regulated environments, cGMP grade. J.T.Baker® Endonuclease Ultrapure Bioreagent, product number BAKR7108, is for use in research and laboratory environments and is available in 100 KU, 1,250 KU, and 5,000 KU.

* J.T.Baker® Endonuclease Ultrapure Bioreagent purity testing is conducted using SDS-Page compared to more sensitive HPLC testing for Biotech Reagent grade with also includes protease testing and acceptance criteria for endotoxin testing.

QUESTION 15: What is the manufacturing capacity and lead-time for J.T.Baker® Endonuclease and do you keep safety stocks in case of supply disruptions?

J.T.Baker® Endonuclease is produced at our Technology and Innovation Center in Bridgewater, New Jersey. This facility can efficiently manufacture multiple batches per month according to customer forecasts. Each batch yields approximately 1,000MU, with the capacity and resources to increase production as needed. The standard lead time is 16 to 24 weeks if the material is not in stock. However, J.T. Baker® Endonuclease is typically made to stock, with minimum safety stocks maintained to prevent supply disruptions.

References

1. Meiss, G. F. (1995). Sequence preferences in cleavage of dsDNA and ssDNA by the extracellular Serratia marcescens endonuclease. *Biochemistry*, 34 - 37.
2. Cygnus® is a registered trademark of Cygnus Technologies, LLC.