

▶ Source

GENIUS™ Nuclease is a recombinant form of *Serratia marcescens* extracellular endonuclease produced in *Escherichia coli* cells using a proprietary process at ACROBiosystems. GENIUS™ Nuclease is a homodimer with monomer molecular masses about 30 kDa. Two disulfide bonds found in the nuclease are crucial to its activity and stability. The enzyme is a non-specific nuclease with high specific activity, which degrades both single- and double-stranded nucleic acids in any form (single stranded, double stranded, linear, circular and supercoiled). It hydrolyzes internal phosphodiester bonds present between the nucleotides to 5'-phosphorylated oligonucleotides of 3-5 bases in length.

▶ Application

Its high intrinsic activity and broad substrate tolerance make the endonuclease an ideal tool in a variety of biotechnological and pharmaceutical applications: removal of nucleic acid from protein samples (Elimination of nucleic acids from recombinant proteins; Purification of protein fragments from inclusion bodies; Sample preparation in western blotting or two-dimensional gel electrophoresis); Viscosity reduction in protein extracts.

▶ Operating conditions

GENIUS™ Nuclease is functional between pH 6 and 10 (optimal at pH 8 - 8.5), and from 0°C to 42 °C (optimal at 35 °C - 42 °C). Mg²⁺ (1-2 mM) is required for enzyme activity. 1 mM EDTA reduced the activity by 30% in the presence of 1 mM MgCl₂; 0.1 M EDTA eliminated all enzyme activity. In the presence of 1 mM MgCl₂, enzyme levels were reduced 75% by 0.1 M CaCl₂ or 1 M NaCl. Under standard assay conditions, 1 mM iodoacetate had no effect on the enzymatic rate, whereas 1 mM mercaptoethanol and maleic acid reduced the activity by only 5 to 10%. 10 mM p-Chloromercuribenzoate completely inactivates the enzyme, while 0.64 M beta-mercaptoethanol in the presence of 2 M urea causes only partial inactivation of the enzyme. 4 or 7 M Urea increases the enzyme activity.

▶ Removal of GENIUS™ Nuclease

GENIUS™ Nuclease contains no "Tag" and used in downstream processing can be removed by various purification methods according to the purification strategy for the target protein.

▶ Formulation

Lyophilized in Tris HCl, pH 8.0, MgCl₂, and NaCl.

▶ Reconstitution

See Certificate of Analysis for reconstitution instructions and specific concentrations.

▶ Purity

>95 % as determined by SDS-PAGE reduced GENIUS™ Nuclease.

▶ Activity Assay Procedure

1. Reagents and solutions preparation

Reaction buffer*:

50 mM Tris-HCl, 1 mM MgCl₂, pH 8.0 (* In the case of extensive dilution before use, carrier protein such as 0.1 mg/ml HSA or BSA is generally recommended to avoid any enzyme loss from surface adsorption)

DNA Substrate:

1 mg/ml salmon sperm DNA is dissolved overnight at 4 °C, in reaction buffer, and is then sonicated on ice to obtain a homogenous solution.

Enzyme:

Different dilution of nuclease with reaction buffer.

Stop reagent:

Trichloroacetic acid (TCA)

2. Standard curve establishment

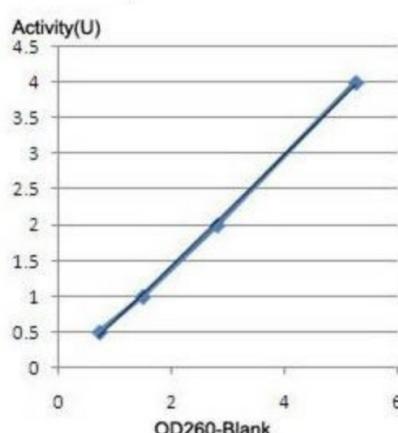
400 µl substrate + 100 µl enzyme of known activity = 500 µl mixture

- Incubate the mixture at 37°C for 30 min.
- Stop the reaction by addition of 400 µl cold TCA and incubate on ice for 10 min.
- Centrifuge at 8500g for 5 min.
- Measure the absorbance of supernatant at 260 nm.
- Lot a standard curve with nuclease of known activities for each set of measurements.

3. Measurement of activity

The activity of any unknown nuclease can be determined from a single measurement by means of the standard curve. The specific activity of GENIUS™ Nuclease is >1.0 x 10⁶ unit/mg protein.

▶ Activity



Standard curve of nuclease activity for GENIUS™ Nuclease.

▶ Unit Definition

One unit will digest sonicated salmon sperm DNA to acid-soluble oligonucleotides equivalent to a ΔA₂₆₀ of 1.0 in 30 min at pH 8.0 at 37 °C, which corresponds approximately to complete digestion of 37 µg DNA. Note that 1 KU=1000 units.

▶ Storage

Avoid repeated freeze-thaw cycles.

This product is stable after storage at:

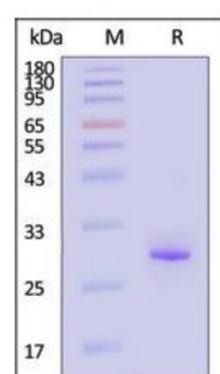
In **lyophilized state** for **1 year** (-20°C);

After **reconstitution** under sterile

conditions for **3 months** (-70°C).

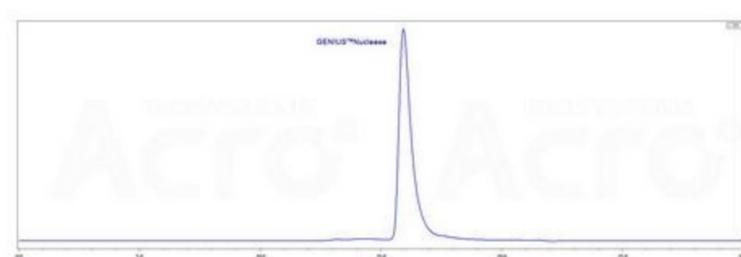
Notice: We updated the brand name from Benz™ Nuclease to GENIUS™ Nuclease. The products are the same and the only change is the brand name in the product name and the label.

▶ 电泳 (SDS-PAGE)



The purity of GENIUS™ Nuclease was determined by SDS-PAGE reduced and staining overnight with Coomassie Blue.

▶ SEC-HPLC



The purity of GENIUS™ Nuclease (Cat. No. BEE-N3116) is more than 95% as determined by SEC-HPLC.

Report