

TurboNuclease™

Cat. No. NU0103P 10,000 units
Cat. No. NU0103S 25,000 units
Cat. No. NU0103M 50,000 units
Cat. No. NU0103L 250,000 units

For Research Use Only

Introduction

TurboNuclease™ is a recombinant form of *Serratia marcescens* extracellular endonuclease (encoded by the same gene of Bezonase) produced in *E. coli* using a proprietary process. This nonspecific endonuclease hydrolyzes both single- and double-stranded nucleic acids (DNA and RNA) to 5'-phosphorylated oligonucleotides of 1-4 bases in length. TurboNuclease™ is a highly purified homodimer of 27 kDa subunits that has exceptional high specific activity and is free of protease activity. TurboNuclease™ is ideal for digesting nucleic acids during virus preparation and for reducing cell lysate viscosity during protein purification.

Activity and Specificity

One unit of TurboNuclease™ converts 1 OD₂₆₀ of salmon sperm DNA into acid-soluble nucleotides in 30 minutes at 37°C in a reaction buffer of 50 mM Tris-HCl, pH 8.0 and 1 mM MgCl₂. This corresponds to complete digestion of 50 µg of salmon sperm DNA into oligonucleotides. TurboNuclease™ has a specific activity of >1.3 x 10⁶ units/mg. This is equivalent to >3 x 10⁶ Kunitz units/mg, over 100-fold specific activity of most highly purified bovine DNase I (~25,000 Kunitz units/mg).

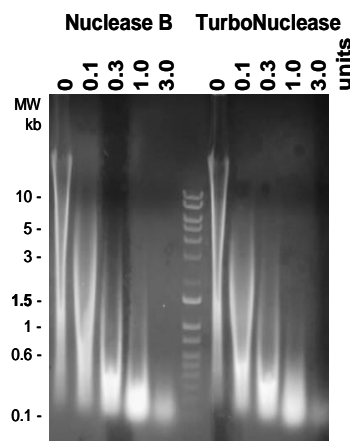


Figure 1

Figure 1

50 µg of salmon sperm DNA was incubated with the indicated units of TurboNuclease™ and another brand of *S. marcescens* endonuclease at 37°C for 30 minutes in a reaction buffer of 50 mM Tris-HCl, pH 8.0 and 1 mM MgCl₂. DNA

digestion was monitored by EtBr-stained agarose gel.

TurboNuclease™ shows no detectable protease activity.

Formulation

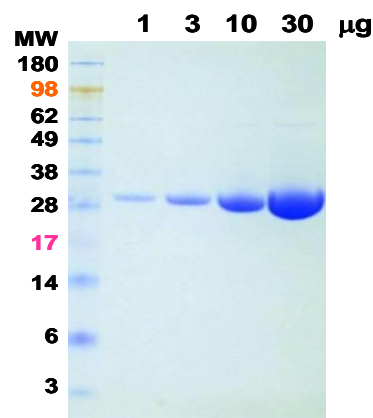


Figure 2

TurboNuclease™ :
 250 units/µl in
 Storage Buffer of 50
 mM Tris-HCl, pH 8.0,
 50 mM NaCl, 5 mM
 MgCl₂ and 50%
 Glycerol
 TurboNuclease™ is
 purified through a
 proprietary process
 that achieves purity

of >99% as shown in Figure 2.

Total endotoxin level is <0.1 EU/1,000 units of TurboNuclease™ as determined by the Endosafe® PTS™ LAL test system.

Storage

Store TurboNuclease™ at -20°C. TurboNuclease™ is stable in the Storage Buffer at 37°C for at least three weeks without any loss of activity.

Application

TurboNuclease™ can be used to reduce viscosity of cell lysates and remove nucleic acid contamination from sample preparations. It reduces smearing when used with 10% SDS to make whole cell lysate for SDS-PAGE. It may reduce or

prevent clumping of concentrated cells and frozen cells following thawing. TurboNuclease™ also replaces crude DNase I in many applications.

To reduce viscosity of cell lysate, 10-500 units of TurboNuclease™ can be used for each gram of cell paste. The efficiency of viscosity reduction may vary with buffers, cell types, and cell lysis methods used. Due to its high specificity, the total amount TurboNuclease™ added is less than 0.1 µg/ml of lysate and will not complicate any downstream process.

Procedures

Large Scale Cell Lysis

1. Make fresh cold Lysis Buffer

Lysis Buffer should be a buffer in which the target protein is soluble. The Lysis Buffer should be compatible with downstream purification processes, e.g. minimal amount of EDTA or DTT if Ni column is used.

Here is an example of Lysis Buffer

25 mM Tris-HCl, pH 8.0

500 mM NaCl

14 mM β-mercaptoethanol

Detergent can be included for less soluble proteins or when protein solubility is unknown. 1% Triton X-100 has no effect on TurboNuclease™ activity. TurboNuclease™ has the same activity in 150 mM NaCl or 500 mM NaCl and 400 mM imidazole.

2. Resuspend thawed cell paste in Lysis Buffer

Use 2-10 ml Lysis Buffer for each gram of cell paste.

TurboNuclease™ can reduce the amount of Lysis Buffer used.

We routinely use 2 ml of lysis buffer for each gram of cell pellets.

3. Add TurboNuclease™ to 25 units/ml

Protease inhibitors can be added at the same time.

If the lysis buffer contains EDTA or EGTA, add 10-fold more TurboNuclease™.

4. Lyse cells by mechanical or chemical methods on ice or at room temperature

TurboNuclease™ also reduces the viscosity of lysate lysed by microfluidizer.

5. Clear lysate by centrifugation for column loading
The reduced viscosity makes it possible to clear the lysate at lower speed. 35,000g (~16,000 rpm) for 1 hour is sufficient.
Lysate can be loaded to "Crude" columns without clearance.

Parallel Lysis of Multiple Insect Cell Samples

1. Freeze cells pellets of 5-10 ml culture on dry ice briefly. Freeze and thaw facilitate lysis.
2. Thaw the frozen pellets and completely resuspend in ~1 ml Lysis Buffer with TurboNuclease™.
3. Transfer the cell suspension to a microtube and sit the tubes on a floater rack.
4. Lyse cells using an Ultrasonic Cleaner with ice waterbath for 10 min.
Ultrasonic Cleaner (many chemists use it) is much cheaper than probe sonicator with the following advantages.

- There is no cross-contamination since each sample is enclosed in a microtube.
- The samples are always cold as long as ice is added in the water-bath.
- There is no limit on the number of samples processed in parallel. A small Ultrasonic Cleaner can easily hold 48 samples.

The lysate can be used for analyses of protein expression of whole cell lysate, soluble lysate, or affinity pull-down.

Lysis Buffer

25 mM Tris-HCl, pH 8.0

500 mM NaCl

20 mM Imidazole, pH 8.0

14 mM β-mercaptoethanol

0.5% Triton X-100

25 units/ml TurboNuclease™

Technical Support

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