Nacalai USA
Innovations for Life Sciences.

# TurboNuclease

**Cat. No. NU0103M** 50,000 units

*For Research Use Only*

## Introduction

TurboNuclease is a recombinant form of *Serratia macescens* extracellular endonuclease (encoded by the same gene of Bezonase) produced in *E. coli* using a proprietary process. This nonspecific endonuclease hydrolyzes both singleand 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 to digest nucleic acids and to reduce viscosity during protein purification and sample preparation.

## Activity and Specificity

One unit of TurboNuclease converts 1 OD260 of salmon sperm DNA into acidsoluble nucleotides in 30 minutes at 37°C in a reaction buffer of 50 mM Tris-HCl, pH 8.0 and 1 mM $MgCl_2$. This corresponds to complete digestion of 50 ug of salmon sperm DNA into oligonucleotides.
TurboNuclease has a specific activity of >$1.3x10^6$ units/mg. This is equivalent to >$3x10^6$ Kunitz units/mg, over 100-fold specific activity of most highly purified bovine DNase I (~25,000 Kunitz units/mg).

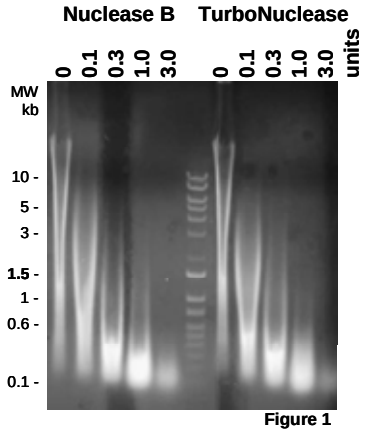

Figure 1

Figure 1
50 ug of salmon sperm DNA was incubated with the indicated units of TurboNuclease and another brand of nuclease at 37°C for 30 minutes in a buffer of 50 mM Tris-HCl, pH 8.0 and 1 mM $MgCl_2$. DNA digestion was monitored by agarose gel. TurboNuclease shows no detectable protease activity.

## Formulation

TurboNuclease: 250 units/ul in Storage Buffer of 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 5 mM $MgCl_2$ 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 LAL Gel-Clot Assay.

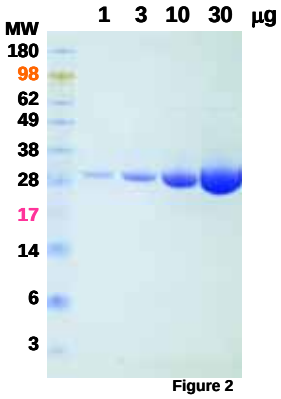

Figure 2

## Storage

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

## Applications

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 ug/ml of lysate and will not complicate any down stream process.

## Procedures

### Lare 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 will be used.

   Here is an example of Lysis Buffer
   25 mM Tris-HCl, pH 8.0
   500 mM NaCl
   14 mM beta-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. Lyze cells by mechanical or chemical methods on ice or at room temperature

TurboNuclease also reduces the viscosity of lysate lyzed by microfluidizer.

5. Clear lysate by centrifugation for column loading
   The reduced viscosity makes it possible to clear the lysate at lower speed. 35,000*g*(~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 facilitates 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. Lyze cells using an Ultrasonic Cleaner with ice waterbath for 10 min
   Ultrasonic Cleaner (many chemists use it) is much cheaper than probe sonicator. It costs a few hundreds US dollars for a new model and less than $100 for a used one or a jewelry cleaner from a consumer goods store. Ultrasonic Cleaner is much better and cheaper than those fancy multi-probe sonicators 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 beta-mercaptoethanol
0.5% Triton X-100
25 units/ml TurboNuclease

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