BioVision

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For research use only

TurboNuclease, Recombinant

CATALOG #: 9207-50KU

AMOUNT: 50,000 units

SOURCE: E. coli

DESCRIPTION: BioVision's TurboNuclease is a recombinant form of *Serratia macescens* extracellular endonuclease (encoded by the same gene of Benzonase) 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.

FORMULATION: 250 units/µl in a storage buffer of 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 5 mM $MgCl_2$ and 50% glycerol

SPECIFIC ACTIVITY: >$1.3x10^6$ units/mg. One unit of TurboNuclease converts 50 µg 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 $MgCl_2$.

ENDOTOXIN LEVELS: < 0.25 EU / 1,000 units of TurboNuclease as determined by the LAL Gel-Clot Assay.

STORAGE CONDITIONS: Store the product at -20˚C. TurboNuclease is stable in the storage buffer at 37˚C for at least three weeks without any loss of activity.

APPLICATIONS: TurboNuclease is very effective in reducing the viscosity of cell lysates. It removes nucleic acid contamination from sample preparations such as Adenovirus and AAV purification. 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. It also replaces crude DNase I in many applications. To reduce viscosity of cell lysate, 10-100 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 of TurboNuclease added is less than 1 µg/ml of lysate and will not complicate any downstream process.

Figure 1

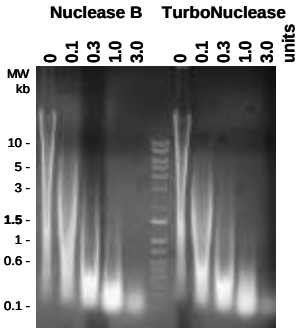

Figure 2

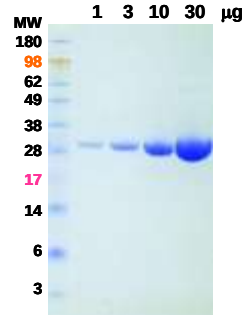

Figure1: 50 µg of salmon sperm DNA was incubated with the indicated units of TurboNuclease and another brand of nuclease at 37 ˚C for 30 min in a buffer of 50 mM Tris-HCl, pH 8.0 and 1 mM $MgCl_2$. DNA digestion was monitored by agarose gel.

Figure 2: TurboNuclease is purified through a proprietary process that achieves purity of >99%.

SUGGESTED PROTOCOL:

A) LARGE SCALE CELL LYSIS:

1. Make a fresh, cold lysis buffer in which the target protein is soluble and is compatible with downstream purification processes, e.g. minimal amount of EDTA or DTT if a Ni-NTA column will be used.

   (An example of lysis buffer would be 25 mM Tris-HCl, pH 8.0, 500 mM NaCl, and 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 the thawed cell paste in lysis buffer. Use 2-10 ml Lysis Buffer for each gram of cell paste (~2 ml of lysis buffer for each gram of cell pellets should work).

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 generated 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.

B) 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 place the tubes on a floater rack.

4. Lyse cells using an ultrasonic cleaner with ice water bath for 10 minutes. The lysate can be used for analyses of protein expression of whole cell lysate, soluble lysate, or affinity pull-down.

FOR RESEARCH USE ONLY! Not to be used on humans.

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