NEBNext® dsDNA Fragmentase®

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M0348S 008140715071

M0348S

50 reactions (500 µl vol) **Lot: 0081407**

RECOMBINANT **Store at –20°C** **Exp: 7/15**

Description: NEBNext dsDNA Fragmentase generates dsDNA breaks in a time-dependent manner to yield 50–1,000 bp DNA fragments depending on reaction time (1). NEBNext dsDNA Fragmentase contains two enzymes, one randomly generates nicks on dsDNA and the other recognizes the nicked site and cuts the opposite DNA strand across from the nick, producing dsDNA breaks. The resulting DNA fragments contain short overhangs, 5´-phosphates, and 3´-hydroxyl groups. The random nicking activity of NEBNext dsDNA Fragmentase has been confirmed by preparing libraries for next-generation sequencing. A comparison of the sequencing results between gDNA prepared with NEBNext dsDNA fragmentase and with mechanical shearing demonstrates that the NEBNext dsDNA Fragmentase does not introduce any detectable bias during the sequencing library preparation and no difference in sequence coverage is observed using the two methods (2).

Source: NEBNext dsDNA Fragmentase is composed of endonucleases isolated from two different *E. coli* sources: one construct expresses a fusion protein consisting of *E. coli* maltose binding protein and *Vibrio vulnificus* nuclease mutant protein; the other expresses a fusion protein consisting of maltose binding protein and T7 endonuclease mutant protein.

Applications:

- Generation of dsDNA fragments for sequencing on next generation sequencing platforms
- Generation of dsDNA fragments for libraries

Supplied in: 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1 mM EDTA, 200 µg/ml BSA, 0.15% Triton X-100 and 50% glycerol.

Updated Components & Protocol

Reagents Supplied with Enzyme:
NEBNext dsDNA Fragmentase
Reaction Buffer v2 (10X)
200 mM $MgCl_2$

Reaction Conditions: 1X NEBNext dsDNA Fragmentase Reaction Buffer v2. Incubate at 37°C.

1X NEBNext dsDNA Fragmentase Reaction Buffer v2:
20 mM Tris-HCl
15 mM $MgCl_2$
50 mM NaCl
0.1 mg/ml BSA
0.15% Triton X-100
pH 7.5 @ 25°C

Reaction Definition: One reaction is defined as the amount of NEBNext dsDNA Fragmentase required to convert 1 µg of purified HeLa cell gDNA in 20 µl of 1X NEBNext dsDNA Fragmentase Reaction Buffer v2 into short (100–300 bp) DNA fragments in 30 minutes at 37°C.

Heat Inactivation: 65°C for 15 minutes in the presence of 50 mM DTT.

Quality Assurance: Free of detectable protease and phosphatase activity.

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection. BSA is added to the enzyme for stability.

Protease Assay: Incubation of 10 µl NEBNext dsDNA Fragmentase with 0.2 nmol of a standardized mixture of proteins, for 20 hours at 37°C, resulted in no proteolytic activity detected by SDS-PAGE.

Phosphatase Assay: Incubation of 10 µl of NEBNext dsDNA Fragmentase in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Protocol:

Digestion with NEBNext dsDNA Fragmentase:

Tip: Adequate mixing of NEBNext dsDNA Fragmentase is important for the success of this reaction. NEBNext dsDNA Fragmentase should be vortexed for 3 seconds prior to use.

For tough digestions, add 1 µl of 200 mM $MgCl_2$ to the reaction. Additional $MgCl_2$ can be added if necessary.

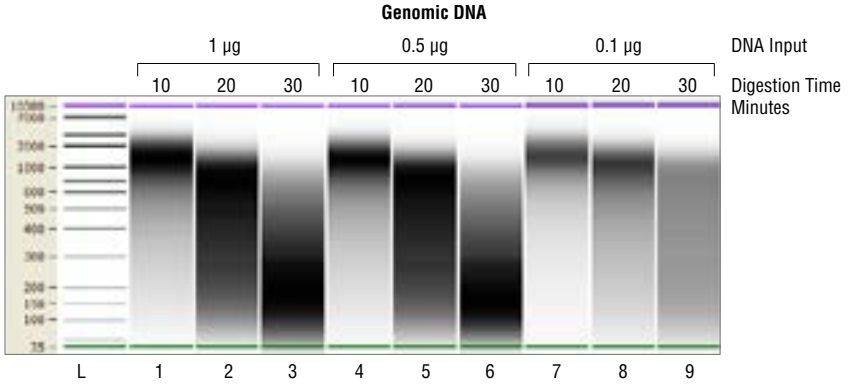

Figure 1: *Fragmentation of E. coli gDNA. E. coli gDNA was fragmented with NEBNext dsDNA Fragmentase for the indicated times and purified on MinElute® columns.*

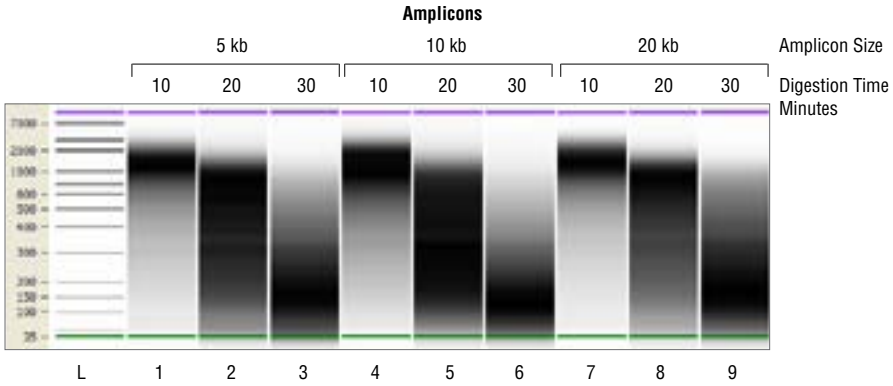

Figure 2: *Fragmentation of 5,10 and 20 kb amplicons with NEBNext dsDNA Fragmentase.*

The protocol listed below is for fragmentation of 5 ng–3 µg of DNA.

1. Vortex NEBNext dsDNA Fragmentase for 3 seconds, quick spin and place on ice.
2. Mix together the following components in a sterile PCR tube:

DNA (5 ng–3 µg)	1–16 µl
10X Fragmentase Reaction Buffer v2	2 µl
Sterile Water	variable
Final Volume	18 µl

3. Add 2.0 µl dsDNA Fragmentase and vortex the mixture for 3 seconds.
4. Incubate at 37°C for the recommended times below to generate the desired fragment size.

Desired Fragment Size (bp)	Incubation Time (min)
50–200	25–35
200–1,000	15–25
1,000–2,000	10–15

*If starting material is 100 ng or less, incubation times should be increased by 10 minutes.

5. Add 5 µl of 0.5 M EDTA to stop the reaction.
6. DNA fragments are ready for DNA end repair, size selection or analysis.

End Repair: Clean up the fragmented DNA (e.g. column purification, or using SPRI) then proceed with desired DNA end repair protocol.

(see other side)

CERTIFICATE OF ANALYSIS

Agarose Gel Size Selection/Analysis: Samples can be loaded directly on to an agarose gel. It is not necessary to clean up the reactions prior to loading.

Polyacrylamide Gel Analysis: Clean up the fragmented DNA (e.g. column purification) prior to loading the samples on a PAGE gel.

Long Term Storage: Clean up the fragmented DNA (e.g. column purifications, or SPRI Beads*) prior to long term storage.

*Note: If using SPRI Beads for sample purification, it is recommended to dilute the sample 1:1 with sterile water to allow for faster collection of beads to the magnet.

References:

1. Patent pending.
2. Unpublished observations.