NEBNext® dsDNA Fragmentase®

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M0348S

50 reactions (500 µl vol) **Lot: 0071301**

RECOMBINANT **Store at –20°C** **Exp: 1/14**

Description: NEBNext® dsDNA Fragmentase® generates dsDNA breaks in a time-dependent manner to yield 100–800 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.

Reagents Supplied with Enzyme:
10X NEBNext dsDNA Fragmentase Reaction Buffer
100X BSA (100 mg/ml)
E. coli DNA Ligase for Fragmentase

Reaction Conditions: 1X NEBNext dsDNA Fragmentase Reaction Buffer, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBNext dsDNA Fragmentase Reaction Buffer:
20 mM Tris-HCl
10 mM $MgCl_2$
50 mM NaCl
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 supplemented with 100 µg/ml BSA 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.

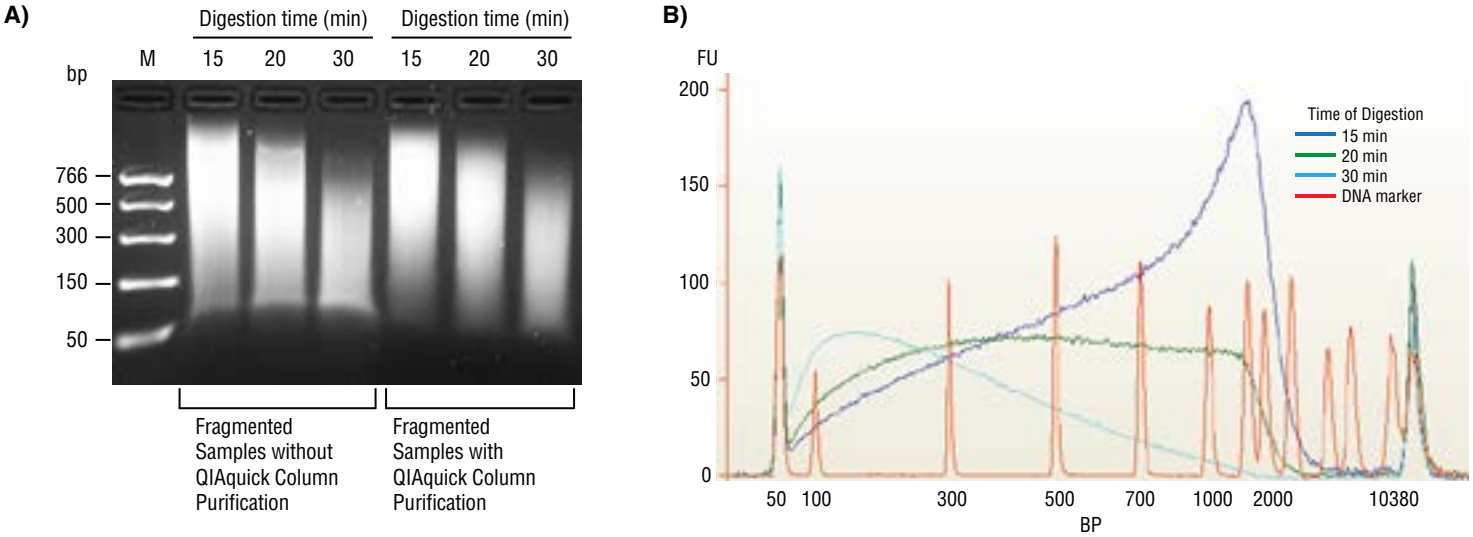

Figure 1: *Fragmentation of HeLa cell gDNA analysed by agarose gel electrophoresis (A) and the BioAnalyzer 2100 (B). 5 µg of genomic HeLa DNA was incubated with NEBNext dsDNA Fragmentase for varying times as indicated. Fragmented DNA was purified using MinElute columns and analyzed by the BioAnalyzer 2100.*

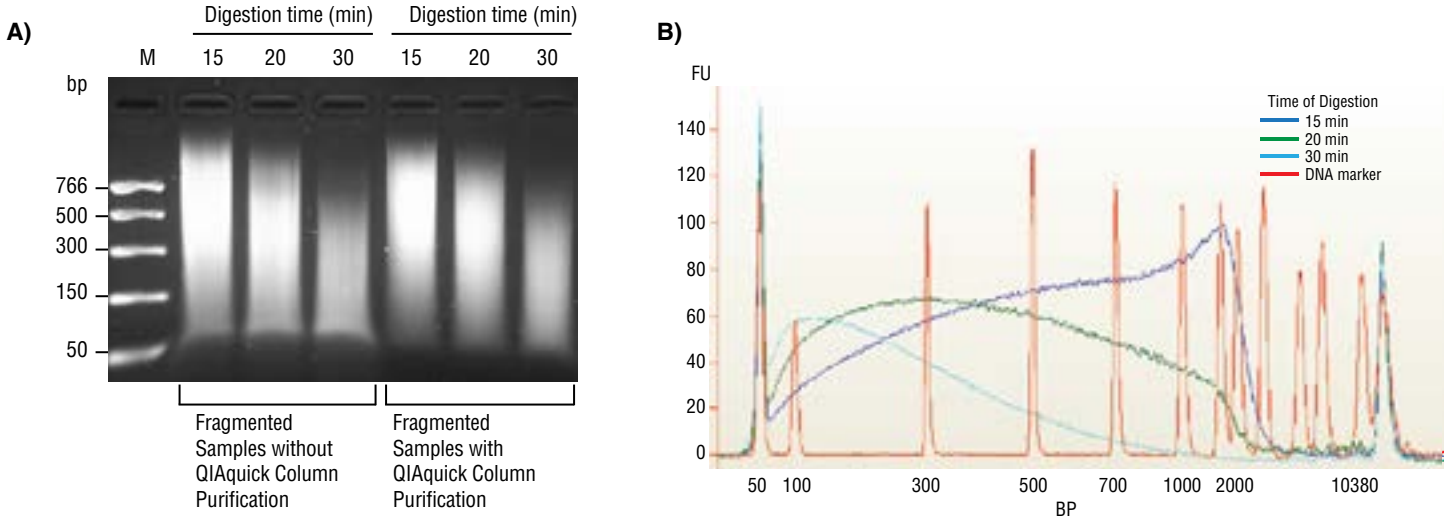

Figure 2: *Fragmentation of E. coli DNA analysed by agarose gel electrophoresis (A) and the BioAnalyzer 2100 (B). 5 µg of genomic E. coli DNA was incubated with NEBNext dsDNA Fragmentase for varying times as indicated. Fragmented DNA was purified using MinElute columns and analyzed by the BioAnalyzer 2100.*

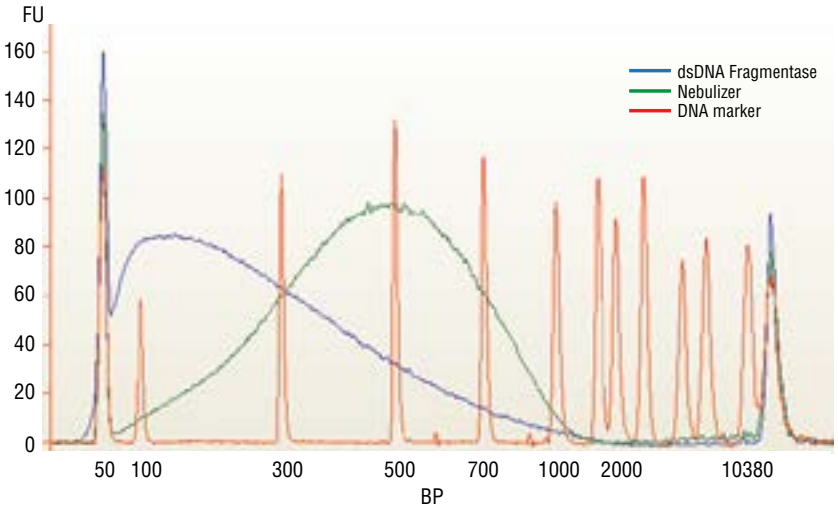

Figure 3: *Relative size distribution of E. coli DNA fragments with NEBNext dsDNA Fragmentase vs. Nebulization as seen using the Bioanalyzer 2100. The dsDNA Fragmentase sample was incubated for 30 minutes at 37°C with 0.5 µg of DNA per µl of NEBNext dsDNA Fragmentase in 1X Fragmentase Reaction Buffer with 100 µg/ml BSA. The Nebulizer sample was prepared by nebulization of DNA in 50% glycerol for 6 minutes at 35 psi.*

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:

Careful preparation and concentration determination of the starting material are important for the success of this reaction.

The recommended range for starting material is 1–5 µg but lower or higher amounts can be used. The final DNA concentration in the reaction should be 0.05 µg/µl. Incubate reaction mix on ice for 5 minutes before adding NEBNext dsDNA Fragmentase. Add 2 µl of NEBNext dsDNA Fragmentase per µg DNA and incubate as described below.

1. Vortex DNA sample.
2. Set up the digestion reaction using the following guidelines **(note that NEBNext dsDNA Fragmentase should not be added at this point)**:

Reaction Components	Starting DNA Amount (µg)				
DNA (µg)	1	2	3	4	5
10X Fragmentase Reaction Buffer (µl)	2	4	6	8	10
100X BSA (µl)	0.2	0.4	0.6	0.8	1.0
dsDNA Fragmentase (µl)	2	4	6	8	10
Final Volume* (µl)	20	40	60	80	100

3. Vortex thoroughly.
4. Incubate on ice for 5 minutes
5. Vortex NEBNext dsDNA Fragmentase and add enzyme to the reaction.
6. Vortex thoroughly.
7. Incubate at 37°C according to the recommended times below to generate the desired fragment size (see notes).

Desired Fragment Size (bp)	Incubation Time (min)
600–800	15
300–600	20
100–300	30

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

End Repair: Clean up the fragmented DNA (e.g. column purification) then proceed with desired DNA end repair protocol. If DNA fragments are to be used for the Illumina® Genome Analyzer DNA sample preparation, add 1 µl of *E. coli* DNA Ligase for Fragmentase to the end repair reaction with ≥ 1 µg DNA or 0.5 µl of *E. coli* DNA Ligase for Fragmentase to the end repair reaction with < 1 µg DNA.

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 purification) prior to long term storage.

Notes: For gDNA containing > 60% GC content, a longer incubation time with dsDNA Fragmentase is required to obtain the desired fragment size. See application note at www.neb.com.

PCR products and cDNA require a different incubation time with dsDNA Fragmentase to obtain the desired fragment size. See application notes at www.neb.com.

The *E. coli* DNA Ligase for Fragmentase contains NAD and is provided only for use in DNA end repair reactions following NEBNext dsDNA Fragmentase incubation and DNA clean up. Do not add *E. coli* DNA Ligase to the NEBNext dsDNA Fragmentase reaction.

References:

1. Patent pending.
2. Unpublished observations.