DNA Fragmentation Kit

Kit for the enzymatic fragmentation of dsDNA

Research Use Only (RUO)

Content

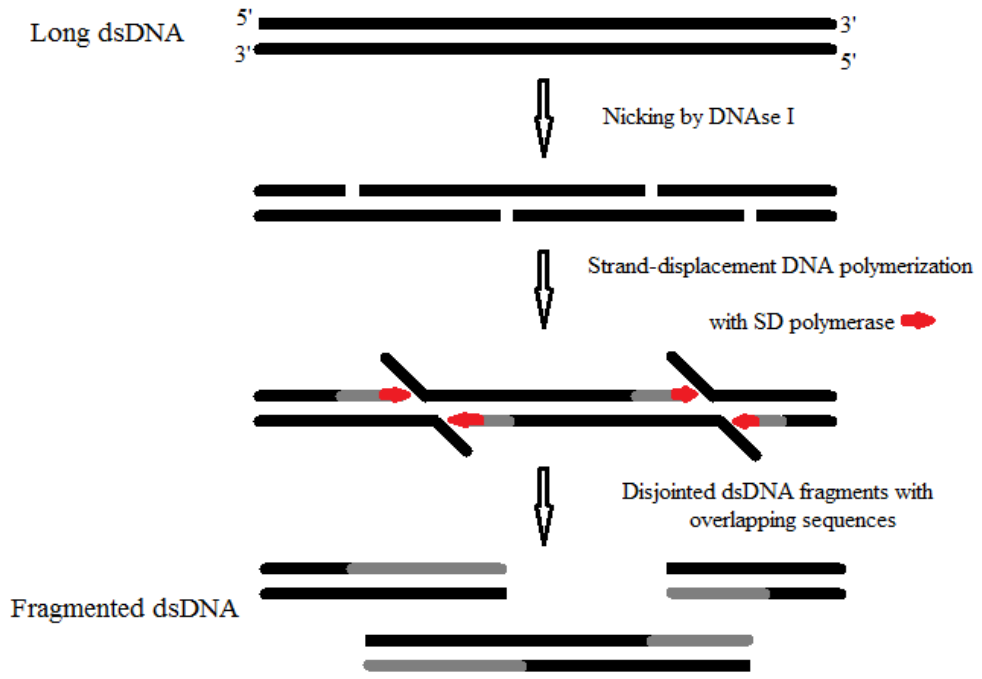
DNA Fragmentation Kit	Ref. No: 131025 (25 preps)
	Ref. No: 131050 (50 preps)
Valid from:	December 2020

1. Introduction

BIORON´S DNA Fragmentation Kit, produced at BIORON GmbH, is designed for enzymatic fragmentation of genomic DNA (100 - 1000 ng) with the formation of double-stranded (ds) fragments of the required length.

The enzymatic fragmentation method used in the kit is based on a combination of two enzymes: nonspecific endonuclease DNase I and SD DNA polymerase with strand displacement activity. SD DNA polymerase is a recombinant Taq DNA polymerase. The enzyme combines the ability to displace DNA strands while maintaining stability at temperatures up to 92 °C and has a maximum level of enzymatic activity at 70–75 °C. At the first stage, DNase I introduces many random single-strand breaks (nicks) into ds DNA. At the second stage, with an increase in the temperature of the mixture, endonuclease DNase I is inactivated, and SD DNA polymerase is activated, which, in turn, fills in the 3'-ends of DNA fragments at the site of a single-stranded break by DNA polymerization with displacement of the leading 5 '→ 3' chain. As a result, many overlapping elongated double-stranded fragments with 3'-A-overhangs at the ends are generated, which makes the fragments suitable for direct ligation with DNA adapters, bypassing the additional steps of repairing the ends of DNA fragments and adenylation.

General overview of the method used in the kit is shown in the picture below (De novo synthesized DNA is indicated in grey and SD polymerase is indicated in red):



The kit includes a reaction buffer, a mixture of enzymes, $MgCl_2$, control DNA (human genomic DNA at a concentration of 1000 ng /µL), magnetic beads for purification of fragmentation products, and water.

2. Content of the Kit

Ref No	XXXX25 25 preps	XXXX50 50 preps	cap color	Storage
Reaction Buffer	100 µl	200 µl	violet	-20 °C
Enzyme Mix	125 µl	250 µl	red	-20 °C
$MgCl_2$	100 µl	200 µl	black	-20 °C
Water	1400 µl	1400 µl	white	-20 °C
Control DNA	50 µl	100 µl	green	-20 °C
Magnetic Beads	750 µl	1500 µl	yellow	+8 °C

Additional Material Required

- PCR Cycler
- PCR tubes suitable for the selected PCR Cycler
- pipette tips with aerosol barrier
- variable-volume single-channel pipettes
- magnetic stand
- 80% ethanol
- safety laminar box
- safety equipment according to your local requirements

3. Storage Conditions and Stability

The components of the Kit (excluding Magnetic Beads) have to be stored at -20 °C and is stable until the expiry date. Magnetic Beads has to be stored at +8 °C.

Thaw the Reaction Buffer, $MgCl_2$, control DNA and water just before use. Keep them at 4 °C and the Enzyme Mix at -20 °C until needed and transport in a cooling rack.

Please avoid freeze of the Magnetic Beads.

Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed.

4. Quality Control

The performance of the **BIORON´S DNA Fragmentation Kit** is monitored routinely on a lot-to-lot basis.

5. Protocol for enzymatic fragmentation

Starting material: purified dsDNA (genomic DNA of humans, animals or plants, bacterial DNA) in an amount of 100 to 1000 ng, dissolved in water.

It is recommended to determine the amount of dsDNA before adding to the reaction mixture by a fluorometric method, for example, using a Qubit instrument.

Before use, the kit components must be completely thawed, mixed by pipetting and left on ice until use.

Reaction setup:

Reaction Buffer	4 µl
Enzyme Mix	5 µl
$MgCl_2$	2.5 - 4 µl*
dsDNA (100 – 1000 ng)	1 - 10
Water	fill up to 25 µl
	25 µl reaction volume

*The range of length of the required fragments depends on the volume of the $MgCl_2$ added to the reaction mixture:

$MgCl_2$ volume	Length of the fragments, b.p.
2.5 µl	300 – 800 (350-600)
3 µl	200 – 600 (275-475)
3.5 µl	150 – 400 (200-350)
4 µl	100 – 300 (150-250)

Fragmentation program:

37 °C	100 min*
70 °C	20 min
10 °C	∞

Attention! The temperature of the heated lid of the PCR cycler should be 80 °C

*Incubation time can vary up and down:

-If the fragments are too long, increase the fragmentation time in steps of 5 - 10 min until the optimal distribution of the fragment length range is achieved

-If the fragments are too short, reduce the fragmentation time in steps of 5 - 10 min until the optimal distribution of the fragment length range is achieved

The fragmentation reaction is stopped by cooling the reaction mixture to 10 °C.

The product of the DNA fragmentation does not require restoration of the ends, and is ready for purification and selection of fragments by length.

6. Reaction cleanup and size selection

The purification protocol is based on the addition of the magnetic beads to the reaction mixture. The volume of added beads is shown in the table below:

Length of the fragments, b.p.	150-250	200-350	275-475	350-600
Volume of the magnetic beads	37.5 µl	30 µl	25 µl	17.5 µl

Cleanup protocol:

1. Mix the magnetic beads thoroughly.
2. Add the specified volume of the magnetic beads to 25 µl of the reaction mixture according to the selected range of fragments length.
3. Mix thoroughly by pipetting.
4. Incubate for 5 minutes at room temperature.
5. Place the tube on the magnetic stand. After the solution is clear (about 5 minutes), carefully remove the supernatant.
6. Add 200 µl of freshly prepared 80% ethanol to the tube in the magnetic stand. Incubate at room temperature for 30 seconds and then carefully discard the supernatant.
7. Repeat step 6.
8. Holding the tube open in the magnetic stand, air dry the magnetic beads for 5 minutes. Attention! Do not overdry magnetic beads! The magnetic beads need to remain dark brown. If the magnetic particles start to lighten, go to the next step immediately.
9. Remove the tube from the magnetic stand. Elute DNA fragments from the magnetic beads by adding 23.5 µl water or 0.1X TE. Mix well and incubate for 2 minutes at room temperature.
10. Place the tube on the magnetic stand. After the solution becomes clear (about 5 minutes), transfer 22.5 µl of the supernatant containing the target fragments into a new tube.

The resulting purified fragments can be used for direct ligation with DNA adapters and further preparation for NGS analysis.

Warranty and Guarantee of Products

The manufacturer guarantees the performance of its **DNA Fragmentation Kit** in the manner described in this handbook. It is up to the purchaser to determine the suitability of **DNA Fragmentation Kit** for its particular use. In case a product fails to perform as warranted by any reason, BIORON´s sole obligation and the customer´s sole remedy is limited to replacement of product free of charge. BIORON excludes all other warranties. We reserve the right to change, alter, or modify our **DNA Fragmentation Kit** to enhance its performance and design. The manufacturer's terms and conditions are available on request.

Limitations of Product Use

The use the product is strictly limited to research purposes only. They are not to be applied for any diagnostic, including human, medical or drug purposes.