Enabling Discovery in Life Science®

BioScore™ Screening and Amplification Kit

for identifying genomic DNAs from formalin-fixed, paraffin-embedded (FFPE) tissue samples that are suitable for array analysis, genomic analysis and other applications

Instruction Manual

Cat. No. ENZ-42440 20 reactions

For research use only.

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I. Overview

Enzo Life Sciences' ***BioScore*™ Screening and Amplification Kit** is a rapid and reliable method for identifying genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples that are suitable for further analysis. The ***BioScore*™** assay is an isothermal whole genome amplification method that can generate more than 10 µg of DNA from 100 ng of high quality genomic DNA in one hour. FFPE DNA samples suitable for further analysis are identified by high yields in the amplification reaction.

The quality of DNA derived from FFPE samples varies widely due to variations in sample handling, fixation, tissue embedding and length of time in storage. Consequently, not all FFPE DNA samples label efficiently or perform adequately in array assays. Such samples are readily identified by the ***BioScore*™ Screening and Amplification Kit**, where DNA quality is scored as ***Poor***, ***Intermediate***, or ***Good***. Samples that amplify in the ***Poor*** range are not suitable for nucleic acid array analysis. ***Intermediate*** or ***Good*** FFPE samples can be directly labeled using either CGH Labeling Kit for BAC Arrays (Cat. Nos. ENZ-42670) or CGH Labeling Kit for Oligo Arrays (Cat. No. ENZ-42671) and assayed for further array analysis with a high probability for acceptable performance. Non-FFPE genomic DNA isolated from fresh or frozen tissue amplifies extremely well and is considered ***Excellent***.

In cases where ***Good*** or ***Excellent*** genomic DNA is in limiting quantities, the ***BioScore*™** kit can be used to amplify the sample DNA to amounts sufficient for labeling by the **Enzo Genomic DNA Labeling System**.

II. Reagents Provided and Storage

The ***BioScore*™ Screening and Amplification Kit** is shipped on dry ice. Upon receipt, store all reagents at –20°C in a nonfrost-free freezer. Avoid repeated freezing and thawing of the **Nucleotide Mix (N)** and **Enzyme** (**E**).

The following reagents are provided in the kit and are sufficient for the amplification of 20 samples.

Reagent	Min. Volume supplied	Vial ID
Primers	400 µL	**P**
Enzyme	20 µL	**E**
Stop Buffer	110 µL	**S**
Nucleotide Mix	200 µL	**N**
Nuclease-free Water	1 mL	**W**

III. Additional Materials Required

- Water bath, heating block or incubator set at 37°C and 99°C
- DNA purification columns (QIAquick PCR Purification Kit, Qiagen No. 28104)
- Reference genomic DNA (Promega Cat. No. G1471 for human male; Cat. No. G1521 for human female)

IV. Recommended Procedures

A. Starting Material

Genomic DNA isolated from any source can serve as template in an amplification reaction in the ***BioScore*™** kit. The concentration of the genomic DNA should be determined by spectrophotometric or fluorometric analysis. The genomic DNA should be free of contaminating RNA. For FFPE tissue samples, the protocol used for isolation of genomic DNA can strongly influence the overall quality and quantity of DNA recovered. A suggested protocol for the isolation is detailed in the Appendix.

B. Amplifying the Genomic DNA

In order to establish the lower and upper limits of the amplification reaction, a negative control reaction (no template DNA) and a positive control reaction (100 ng of ***Excellent*** high quality, non-FFPE genomic DNA) should be included in the protocol. We recommend amplifying the same reference DNA that will be labeled for array analysis (*e.g.,* pooled normal male or pooled normal female from Promega).

1. Combine 100 ng (up to 19 µL) of genomic DNA (FFPE-extracted or non-FFPE) with 20 µL **Primers** (**P**) and add enough **Nuclease-free Water** (**W**) to bring the reaction mixture to 39 µl. Mix by gently flicking tube, centrifuge briefly.
2. Heat at 99°C for 10 minutes. Place tubes immediately on ice for 5 minutes. Centrifuge briefly and return to ice.
3. While on ice, add 10 µl **Nucleotide Mix** (**N**) and 1 µL **Enzyme** (**E**). Mix by gently flicking the tube. Centrifuge briefly and incubate at 37°C for 1 hour.
4. Add 5 µL of **Stop Buffer** (**S**). Mix by gently flicking the tube. Centrifuge briefly and keep at room temperature.

C. Purifying the Amplified DNA

NOTE: *In order to avoid exceeding the binding capacity of the QIAquick purification column, we recommend that only one-half of the amplification reaction with* ***Excellent/Reference*** *DNA be subjected to purification. The theoretical yield is therefore twice the measured amount.*

1. Use the QIAquick PCR Purification Kit. Add 300 µL of PB Buffer (≥ 5 volumes) to each tube. Mix thoroughly by repeated inversion and centrifuge briefly. Add each sample to a separate QIAquick column. Centrifuge at 13,000 RPM (16,000 x *g*) for 1 minute. Discard filtrate.
2. Add 700 µL of Buffer PE. Centrifuge at 13,000 RPM (16,000 x *g*) for 1 minute. Discard filtrate.
3. Add 700 µL of Buffer PE. Centrifuge at 13,000 RPM (16,000 x *g*) for 1 minute. Discard filtrate. Transfer the QIAquick column to a new collection tube. Centrifuge again at 13,000 RPM (16,000 x *g*) for 1 minute. Transfer the QIAquick column to a new 1.5 mLmicrofuge tube.

4. Add 25 µL of Buffer EB (preheated to 37°C). Incubate at RT for 1 minute. Elute the amplified DNA by centrifugation at 13,000 RPM (16,000 x *g*) for 1 minute. Add an additional 25 µL of Buffer EB (preheated to 37°C). Incubate at RT for 1 minute. Centrifuge at 13,000 RPM (16,000 x *g*).
5. Measure concentration of the amplified DNA by spectrophotometric analysis and calculate yield.
6. Store amplified products at 4°C for short-term storage (less than a week) and at –20°C for long-term storage.

D. Interpreting the Results of the Amplification Reaction

The table below indicates the DNA yields expected from a 1 hour amplification of 100 ng of input DNA. These values are not applicable for reactions longer than 1 hour or with inputs greater than or less than 100 ng. Samples with yields in the ***Poor*** range are unlikely to yield informative array results and should not be used.

DNA Quality	Total Yield (µg)
No input	< 0.5
Poor	< 1
Intermediate	1-3
Good	3-10
Excellent	> 10

The exact yields used for scoring DNAs may vary slightly depending on the user's labeling conditions and array platform.

E. Labeling of Array Asays

1. Direct Labeling of Unamplified DNA

   - 850 ng of ***Good*** genomic DNA can be used for direct labeling and is likely to yield excellent array results.
   - >850 ng of ***Intermediate*** genomic DNA should be used for direct labeling (*e.g.,* 1.7µg).
   - ***Poor*** input DNA should not labeled.

2. Labeling of Amplified DNA

   - 850 ng of amplified DNA from 100 ng of genomic sample DNAs scored as ***Excellent*** or ***Good*** can be labeled for successful array analysis.
   - 850 ng of amplified DNA from from 100 ng of genomic sample DNAs scored as ***Intermediate*** can be labeled for array analysis. These arrays, however, are likely to have higher noise levels than arrays using unamplified DNA.

   NOTE: *The reference DNAs must be amplified by the identical procedures used for the sample DNAs.*

V. Appendix

ISOLATION OF GENOMIC DNA FROM FFPE TISSUE SAMPLES

A. Additional Materials Required

- Reagents for genomic DNA extraction from FFPE tissue sections (QIAamp DNA Mini Kit, Qiagen No. 51304; xylene or xylene substitute; methanol; 100% ethanol; 1M NaSCN)
- Eppendorf Thermomixer or equivalent *(optional)*

B. Recommended Procedure

Day 1: Tissue Treatment

1. Cut three or four 50 mm sections from FFPE tissue blocks and place in a 1.5 mL microcentrifuge tube. Add 1 mL of Xylene, mix thoroughly by repeated inversion, and incubate for 7 minutes at room temperature (RT). Centrifuge at RT for 5 minutes at maximum speed. Remove supernatant. Repeat 1mL xylene wash two more times.
2. Add 1 mL of methanol and mix thoroughly by repeated inversion. Incubate at RT for 5 minutes. Centrifuge at RT for 5 minutes at maximum speed. Remove supernatant. Repeat methanol wash.
3. Add 1 mL of 100% ethanol and mix thoroughly by repeated inversion. Centrifuge at RT for 5 minutes at maximum speed. Remove supernatant.
4. Add 1 mL of 96% ethanol and mix thoroughly by repeated inversion. Centrifuge at RT for 5 minutes at maximum speed. Remove supernatant.
5. Add 1 mL of 70% ethanol and mix thoroughly by repeated inversion. Centrifuge at RT for 5 minutes at maximum speed. Remove supernatant.
6. Add 1 mL nuclease-free water and mix thoroughly by repeated inversion. Centrifuge at RT for 5 minutes at maximum speed. Remove supernatant.
7. Add 1 mL of 1M NaSCN and mix thoroughly by repeated inversion. Incubate overnight at 38°C.

Day 2: DNA Isolation

1. Centrifuge at RT for 5 minutes at maximum speed. Remove supernatant.
2. Add 1 mL of PBS and mix thoroughly by repeated inversion. Centrifuge at RT for 5 minutes at maximum speed. Remove supernatant. Repeat PBS wash.
3. Add 200 µL of Buffer ATL (Qiagen QIAamp Kit) and 20 µL of Proteinase K (Qiagen, 20 mg/mL). Mix thoroughly by repeated inversion and briefly centrifuge. Incubate all day at 55°C with shaking at 500 rpm (Eppendorf Thermomixer). Alternatively, occasional mixing by inversion can be performed.
4. Add 20 µL of Proteinase K at the end of the day and continue incubation overnight at 55°C.

Day 3/Day 4: DNA Isolation (Continued)

1. Add an additional 20 µL of Proteinase K. Mix thoroughly by repeated inversion and briefly centrifuge. Incubate at 55°C with shaking until tissues are completely dissolved. If tissues are not dissolved by the end of Day 3, add another 20 mL of Proteinase K and incubate overnight until the morning of Day 4.
2. Add 4 µL of 100 mg/mL RNase A (Qiagen). Mix thoroughly by repeated inversion and briefly centrifuge. Incubate for 2 minutes at RT.
3. Add 400 µL of Buffer AL, mix thoroughly by repeated inversion and briefly centrifuge. Incubate for 10 minutes at 70°C. Briefly centrifuge.
4. Add 420 µL of 100% ethanol. Mix thoroughly by repeated inversion. Transfer half of the sample to a QIAamp Spin Column in a 2 mL collection tube. Centrifuge for 1 minute at 8,000 RPM (6,000 x *g*). Discard filtrate.
5. Add remainder of sample to the spin column and centrifuge for 1 minute at 8,000 RPM (6,000 x *g*). If the sample does not pass completely through the spin column, repeat centrifugation at higher speed until all of the sample has passed through. Place the spin column in a new 2 mL collection tube and discard the filtrate containing tube.

6. Add 500 µL of Buffer AW1 to the spin column. Centrifuge for 1 minute at 8,000 RPM (6,000 x *g*). Place the spin column in a new 2 mL collection tube and discard the filtrate containing tube.

7. Add 500 µL of Buffer AW2 to the spin column. Centrifuge for 3 minutes at maximum speed. Transfer the column to new microcentrifuge tube and discard the filtrate containing tube. Centrifuge for 1 minute at maximum speed. Transfer the column to a new microcentrifuge tube.

8. Add 75 µL of Buffer AE (preheated to 70°C) to the spin column. Incubate at RT for 1 minute and elute by centrifugation for 1 minute at 8,000 RPM (6,000 x *g*). Repeat elution with another 75 µL of preheated Buffer AE. Store at 4°C. For long-term storage, aliquot genomic DNA and maintain at -20°C.

9. Quantify genomic DNA by spectrophotometric analysis. Evaluate size distribution by agarose gel electrophoresis. DNA quality will by determined by the amplification reaction.

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