PROTOCOL FOR USING
BioMarker® EXT

Introduction: Following the amplification of DNA by PCR, there is a need to determine if the appropriate product has been produced. Typically, this is accomplished by electrophoresis and comparison of product bands to the bands of a restriction digested DNA marker such as *Hinc* II or *Hae* III ΦX174 digests. It is not uncommon for the bands observed in the market lane to have ambiguities arising from comigration of bands having similar lengths of base pairs (bp). BioMarker® EXT is a product designed to give a series of specific bands consisting of 2000, 1500, 1000, 700, 525, 500, 400, 300, 200, 100, and 50 base pairs. The bands observed with BioMarker® EXT are of equal intensity and of unambiguous base pair number. Consequently, BioMarker® EXT standards permit a more accurate determination of PCR* product length than existing markers.

Materials and Equipment:

Kit Components:
1. BioMarker® EXT-Ready to use stock (250µl-50 Assays. Store at 4°C).
2. BioTracker™ 6X Gel Loading Buffer (Store at 4°C or 25°C).

Other Components:
3. Reagents and accessories normally used for DNA electrophoresis.

General: It is assumed that the user is familiar with the electrophoresis of DNA in polyacrylamide and agarose gels and the staining of these gels with ethidium bromide and that they are familiar with the safe use of the equipment and materials employed in these procedures. A convenient reference for these techniques is to be found in *Molecular Cloning: A Laboratory Manual* by J. Sambrook, E.F. Fritsch, and T. Maniatis, Cold Spring Harbor Laboratory Press, 1989.

Procedure:

1. Combine 5µl sample and 1µl of 6X gel loading buffer. If different volumes are required, maintain these ratios.
2. Combine 5µl of BioMarker® EXT with 1µl of 6X gel loading buffer.
3. Load samples on gel applying BioMarker® EXT to either the extreme right hand, extreme left hand, or both lanes of gel.
4. Following electrophoresis, stain and photograph the gel per your usual protocol.
5. Measure and read the distance each band of DNA has traveled down the lane from the bottom of the application well.
6. If more precise estimates of PCR product size are desired, plot the migration distance of the BioMarker® EXT standards on graph paper. Note there are eleven bands appearing in the following order from the origin: 2000, 1500, 1000, 700, 525, 500, 400, 300, 200, 100, and 50 on the gel. Base pairs corresponding to the BioMarker® EXT bands are preprinted on the Y-axis. The corresponding distances can be appropriately scaled and represent the X-axis.
7. After connecting the data points corresponding to the BioMarker® EXT standards, plot the migration distance of the specimens and read their length in base pairs from the Y-axis recording your results.
8. Alternately, the migration distances of the standards can be entered into software which results in calculated values of band length in base pairs.
9. For better accuracy of base pair length determinations a sample+ BioMarker® EXT, a sample- BioMarker® EXT, and a BioMarker® EXT only specimen can be run side by side and the base pair length of the sample band can be determined as described above.

Performance Characteristics: When compared against PCR products of known sequence, BioMarker® EXT has provided molecular weight determinations of the products within 5 base pairs of their known values for products over the range of 2000-50 base pairs.

Obviously, the reliability of the method depends on the degree of accuracy with which the migration bands can be read on the gel photograph; consequently, the greater resolution of the photograph, the greater accuracy in reading the migration distances.

Eurogentec Headquarters
LIEGE SCIENCE PARK • 4102 SERAING • Belgium • Tel: +32 4 372 76 65
Fax: +32 4 372 75 00 • info@eurogentec.com • www.eurogentec.com
Eurogentec North America
3347 Industrial Ct • Suite A • San Diego, CA 92121 • USA • Tel: +1 858 793 26 61
Fax: +1 858 793 26 66 • info.usa@eurogentec.com • www.eurogentec.com

Eurogentec

TIPS FOR RUNNING AGAROSE GELS

I. One of the most common mistakes in running agarose gels is making the gel too thin or making the wells in the gel too shallow.

A. Optimum width for the teeth of the comb used to make the wells is 4-5mm.
B. Comb thickness should be 1.5mm.
C. Optimum thickness of gel is 4-5mm as measured from center as opposed to the edge of the gel. Teeth of comb should be no more than 1.5mm from the bottom of the gel so that the wells themselves are from 2.5-3.5mm deep. This will prevent diffusional loss of samples through the top of the gel as the DNa migrates through the gel.

II. It is extremely important to use the right percentage of agarose, to run it at the proper voltage and for a suitable length of time.

For a mini-gel of 6.5 X 8cm:

A. For resolving 50bp-1000bp, we suggest a 4% agarose consisting of 3% NuSieve GTG agarose and 1% some other low melting temperature agarose. 200V for around 30 minutes should give good resolution in this range. Use BioMarker® Low in this range.
B. For 1000-3000bp, 2% low melting temperature agarose is optimum. 50V for 3-4 hours will resolve this range well. Use BioMarker® High for this bp range.
C. For 1000bp-4361bp, 0.5% low melting temperature agarose will give good resolution. 25V for 3-4 hours will resolve this range well. Use CloneCheck for this bp range.

FOR LARGER GEL DIMENSIONS, RUNNING TIMES WILL BE LONGER AND VOLTAGE MAY NEED TO BE ADJUSTED.

PRIMARY PROTOCOL FOR RADIOLABELING BIOMARKER® WITH ^{32}P

1. Concentrate 25µl of BioMarker® Low in a Microcon-30® or equivalent microconcentrator following manufacturer's instructions.
2. Wash 2 X 25µl with sterile DIH_2O.
3. Backspin for 2 minutes at 2,000 x g.
4. Add sterile DIH_2O to bring back to a 25µl volume.
5. Combine the following:
   - 5µl BioMarker®
   - 5µl T4 kinase buffer (if 5X)
   - 5µl T4 sterile DIH_2O
   - 5µl T4 kinase
   - 5µl gamma ^{32}P (ATP)

   25µl Total Volume
6. Incubate at 37°C for 30 minutes.
7. Incubate at 55°C for 10 minutes to deactivate enzymes.
8. Centrifuge for 5 minutes at 10,000 x g.
9. Dilute 1µl each of the above solution to ratios of: 1:2, 1:5, 1:10, and 1:20.
10. Load 3-4µl of each dilution on gel.
11. Choose the dilution with the optimal results as to exposure time and image quality.

CERTIFIED QUALITY SYSTEM ISO 9002 AV AIB VINCOTTE INTERNATIONAL

Eurogentec Headquarters
LIEGE SCIENCE PARK • 4102 SERAING • Belgium • Tel: +32 4 372 76 65
Fax: +32 4 372 75 00 • info@eurogentec.com • www.eurogentec.com
Eurogentec North America
3347 Industrial Ct • Suite A • San Diego, CA 92121 • USA • Tel: +1 858 793 26 61
Fax: +1 858 793 26 66 • info.usa@eurogentec.com • www.eurogentec.com