Eurogentec

PROTOCOL FOR USING
BioMarker® 10KB

Description: The BioMarker® 10KB DNA ladder is composed of linear double stranded DNA which has been predigested with alkaline phosphatase to expose free OH groups for convenience in kinasing the marker. The marker is suitable for sizing and quantitative estimates over the range of 1kb-10kb for linear double stranded DNA's.

Materials and Equipment:

Kit Components:

1. Two tubes 250µl (each) of BioMarker® 10KB having 9 bands corresponding to 1, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.0, and 10kb of linear double stranded DNA each at 10µg/ml and total DNA of 90µg/ml.
2. 250µl of BioTracker™ 6X Gel Loading Buffer (Store at 4°C or 25°C) and tracking dye.

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® 10KB with 1µl of 6X gel loading buffer.
3. Load samples and BioMarker® 10KB on gel.
4. Perform electrophoresis per your required protocol.
5. Following electrophoresis, stain and photograph the gel per your usual protocol.
6. Determine sample DNA sizes by comparison of migration distances relative to the bands in the BioMarker® 10KB standard.

Performance Characteristics: When processed as set forth above, the marker will give 9 bands of equal intensity by ethidium bromide staining of excellent clarity with only background ethidium bromide between the bands.

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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.

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