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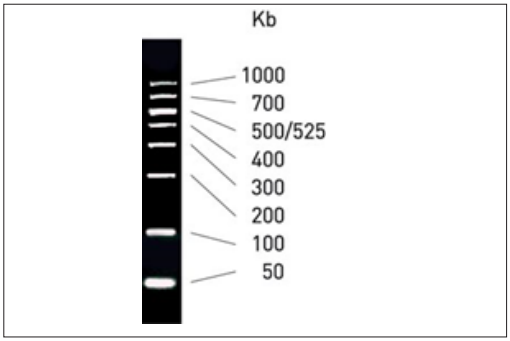
BioMarker® Low
MW-0200-L • MW-0200-LB • MW-0200-LR

Eurogentec products are sold for research or laboratory use only and are not to be administrated to humans or used for medical diagnostics.
BioMarker™ is a range of optimal ladders for reading DNA fragments ranging from 50 to 10000 bp. They are stable at room temperature and are supplied dephosphorylated in order to allow labeling with polynucleotide kinase onto free 5'-termini. Biotin and Rhodamine labeled biomarkers are also available.
Biomarker Low contains 8 linear double-stranded DNA bands; 50, 100, 200, 300, 400, 500, 525, 700 and 1000 bp.



The concentration of each band is 50 ng/ 5 µl of Biomarker Low applied.

Package content

Product	Catalog#	Content
BioMarker® Low	MW-0200-L	BioMarker® Low-Ready to use stock (250µl-50 Assays)
BioMarker® Low biotinylated	MW-0200-LB	
BioMarker® Low rhodamine	MW-0200-LR	BioTracker™ 6X Gel Loading Buffer

Storage Condition
Store BioMarker® Low at 4°C.
Store BioTracker™ 6X Gel Loading Buffer at 4°C or 25°C.

Instruction for Use
1. Combine 5µl sample and 1µl of 6X gel loading buffer. If different volumes are required, maintain these ratios.
2. Combine 5µl of BioMarker® Low with 1µl of 6X gel loading buffer.
3. Load samples on gel applying BioMarker® Low 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® Low standards on 2 cycle semi-log graph paper.

   Note there are nine bands appearing in the following order from the origin: 1000, 700, 525, 500, 400, 300, 200, 100, and 50 on the gel. Base pairs corresponding to the BioMarker® Low 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® Low 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 with BioMarker® Low, a sample without BioMarker® Low, and a BioMarker® Low 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® Low has provided molecular weight determinations of the products within 5 base pairs of their known values for products over the range of 1000-50 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

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resolution of the photograph, the greater accuracy in reading the migration distances.

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:
For resolving 50bp-1000bp, we suggest a 2% Agarose Small Fragments (Cat# EP-0020-10), 200V for around 30 minutes should give good resolution in this 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 DIH2O 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 32P (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.

For further information please contact our Customer Help Desk:

e-mail: info@eurogentec.com
Tel.: 00 800 666 00 123 (European toll free number)