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SmartLadder MW-1700-10

Eurogentec products are sold for research or laboratory use only and are not to be administered to humans or used for medical diagnostics.

The SmartLadder is a popular ready-to-use molecular weight marker, especially designed for easy DNA quantification as well as size determination.

Package content

Reagent	Volume	Description
SmartLadder 1000 lanes	5 x 1000 µl	5 tubes of 200 lanes each, ready to use

Shipping conditions

Shipped at ambient temperature. For long term storage, freeze upon arrival.

Storage

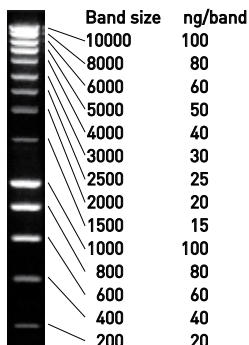
The SmartLadder can be stored at room temperature for 1 month or at 4 °C for 6 months. For long-term storage keep at -20 °C. Avoid multiple freeze-thaw cycles.

Size range

14 spaced bands from 200 to 10 000 bp. The 1000 and 10000 bp bands have a higher intensity than the others to allow quick and easy identification. The size of each band is an exact multiple of 100 bp.

Quantification

Using a standard loading of 5 µl (total amount 720 ng), each band corresponds to an exact quantity of DNA, from 15 to 100 ng.



Loading Buffer composition

> Bromophenol blue	0.25 g/l
> Xylene cyanol	0.25 g/l
> Ficoll 400	25 g/l
> Sodium Azide	1 g/l
> Chloroform	1/1000
> TE (Tris 10mM, EDTA 1mM, pH 8)	

Recommended Procedure

1. Vortex the ladder gently to ensure the solution is homogenous
2. Apply approximately 5 µl per 5 mm lane width.



Do not heat before loading.

T4 DNA Polymerase Labelling Protocol

1. *Exonuclease Reaction (Degradation of DNA from both 3'-ends)*

> To a 1.5 ml microcentrifuge tube on ice, add the following:

– 5X T4 DNA polymerase reaction buffer	4 µl
<i>(165 mM Tris acetate (pH 7.9), 330 mM sodium acetate, 50 mM magnesium acetate, 2.5 mM DTT, 500 µg/ml BSA)</i>	
– SmartLadder	10 µg
– T4 DNA polymerase	40 units
– Autoclaved water	to 20 µl

- > Mix the tube thoroughly but not vigorously
- > Centrifuge briefly
- > Incubate 2 min at 37 °C (about 25 nucleotides/min are removed)
- > Cool reaction vial on ice

2. *Resynthesis Reaction (Resynthesis of the degraded DNA strands)*

- > Add into the reaction vial the following components:

– Autoclaved water	8 µl
– 5X T4 DNA polymerase reaction buffer	6 µl
– dCTP (2 mM)	5 µl

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- dGTP (2 mM) 5 µl
- dTTP (2 mM) 5 µl
- [α -³²P]dATP (3000 Ci/mmol; 10 mCi/ml) 1 µl

- > Mix thoroughly
- > Centrifuge briefly
- > Incubate 2 min at 37 °C
- > Add 5 µl of 2 mM dATP
- > Incubate 2 min at 37 °C
- > Stop reaction by adding 2.5 µl of 0.5 M EDTA
- > Centrifuge for 10 s
- > The cpm incorporated is determined by adding 1 µl of reaction to 24 µl of 250 mM NaCl, 25 mM EDTA
- > Spot 5 µl of dilution on a glass fiber filter
- > Place filter in 10 % (w/v) TCA + 1 % (w/v) pyrophosphate.
- > Wash filter 3 times with 5 % (w/v) TCA and then 2 times with ethanol
- > The filter is dried and then counted using an appropriate scintillant
- > Add 5 µl 0.1 % (w/v) bromophenol blue, 0.1 mM EDTA, 50 % (v/v) glycerol to the sample
- > Load 1 x 10⁵ cpm in a lane.

5' DNA Terminus Labelling Protocol (Phosphate Exchange Reaction)

This reaction will yield specific activities of approximately 1-5 x 10⁵ cpm/pmol of ends.

T4 polynucleotide kinase



- > Add the following components to a 0.5 ml microcentrifuge tube in the following order:
 - Autoclaved water 11 µl
 - SmartLadder 5 µg

- 5X exchange reaction buffer 5 µl
(250 mM imidazole (pH 6.4), 1.5 mM ADP, 60 mM MgCl₂, 75 mM, 2-mercaptoethanol)
- [γ -³²P]ATP (10 µCi/µl) 3 µl
- T4 polynucleotide kinase (5 or 10 U/µl) 1 µl
- > Incubate the reaction mixture at 37 °C for 30 minutes.
Increasing reaction times beyond 30 min will not increase labeling of the DNA.
- > Stop reaction by adding 1 µl of 0.5 M EDTA.
- > Centrifuge for 10 s.
- > Determine radioactive incorporation as above.
- > Add 5 µl 0.1 % (w/v) bromophenol blue, 0.1 mM EDTA, 50 % (w/v) glycerol to the sample.
- > Load 1 x 10⁵ cpm in a lane.

Related products

Reagent	Package size	Reference
Smart Ladder SF	400 lanes	MW-1800-04
Agarose Molecular Biology Grade	100 g	EP-0010-01
	500 g	EP-0010-05
	1 Kg	EP-0010-10
Agarose AgaTabs	300 tablets	EP-0030-15
Mupid®-One Electrophoresis system	1	MU-0041

For further information please contact our Customer Help Desk:

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