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SmartLadder SF
MW-1800-04

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

Descriptions

The SmartLadder SF is a ready-to-use molecular weight marker, especially designed for easy quantification and size determination of short DNA fragments.

Package content

Reagent	Volume	Description
SmartLadder SF 400 lanes	2 x 1 ml	2 tubes of 200 lanes each, ready to use

Shipping conditions

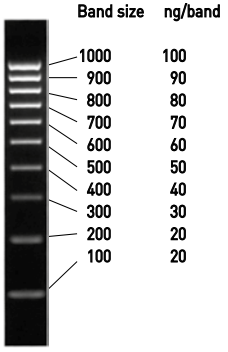
Shipped at room temperature. For long term storage, freeze upon arrival.

Storage

The SmartLadder SF is stable for 1 month at RT or at 4 °C for 6 months. For long-term storage keep at -20 °C. Avoid multiple freeze-thaw cycles.

Size range

The SmartLadder SF produces a pattern of 10 regularly spaced bands ranging from 100 to 1000 bp. All bands have a different intensity to allow a quick and easy identification. The size of each band is an exact multiple of 100 bp.



Quantification

Using a standard loading volume of 5 µl, each band corresponds to an exact quantity of DNA, from 20 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 an homogenous solution.
2. Apply approximately 5 µl per 5 mm lane width.

Do not heat before loading.

Additional protocols:

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 *(165 mM Tris acetate (pH 7.9), 330 mM sodium acetate, 50 mM magnesium acetate, 2.5 mM DTT, 500 µg/ml BSA)*	4 µl
– 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
– dGTP (2 mM)	5 µl
– dTTP (2 mM)	5 µl
– [α-^{32}P]dATP (3000 Ci/mmol; 10 mCi/ml)	1 µl

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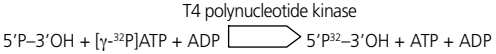
> Mix thoroughly
> Centrifuge briefly
> Incubate 2 min at 37 °C
> Add 5 µl of 2 mM dATP
> Incubate 2 min at 37 °C
> Stop reaction by adding 2.5 µl of 0.5 M EDTA
> Centrifuge for 10 s
> The cpm incorporated is determined by adding 1 µl of reaction to 24 µl of 250 mM NaCl, 25 mM EDTA
> Spot 5 µl of dilution 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 10^5 cpm in a lane.

5' DNA Terminus Labelling Protocol (Phosphate Exchange Reaction)

This reaction will yield specific activities of approximately 1-5 x 10^5 cpm/pmol of ends.



> 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 $MgCl_2$, 75 mM, 2-mercaptoethanol)
– [γ-^{32}P]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 10^5 cpm in a lane.

Related products

Reagent	Package size	Reference
Smart Ladder	1000 lanes	MW-1700-10
Agarose Molecular Biology Grade	100 g 500 g 1 Kg	EP-0010-01 EP-0010-05 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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TDS-MW-1800-04-0810-V2