Low Range ssRNA Ladder

NEW ENGLAND BioLabs Inc.
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N0364S 0191204130 41

N0364S

25 gel lanes (25 µg) **500 µg/ml** **Lot: 0191204**
Store at –70°C **Exp: 4/13**

Description: The Low Range ssRNA Ladder is a set of 6 RNA molecules produced by *in vitro* transcription of a mixture of 6 linear DNA templates. The ladder sizes are: 1000, 500, 300, 150, 80 and 50 bases. The 300 base fragment is at double intensity to serve as a reference band. This ladder is suitable for use as an ssRNA size standard on denaturing polyacrylamide-urea gels, and on denaturing or native agarose gels.

Supplied in: 20 mM KOAc (pH 4.5)

Usage Recommendation: This marker was not designed for precise quantification of ssRNA mass.

Note: Store at –70°C. For short term storage (< 1 week), ladder can be stored at –20°C.

Reagents Supplied with Ladders:
2X ssRNA Ladder Loading Buffer
(for use with denaturing polyacrylamide-urea gels and native agarose gels)

2X ssRNA Loading Buffer:
2X TBE (pH 8.3)
13% ficoll (w/v)
0.01% bromophenol blue
7 M urea

Note: Buffer **no** longer contains xylene cyanol ff.

Denaturing vs. Native Agarose Gels: It is common practice to electrophorese RNA on a fully denaturing agarose gel, such as one containing formaldehyde (1). However, in many cases it is possible to run RNA on a native agarose gel and obtain suitable results. In fact, it has been demonstrated that treatment of RNA samples in a denaturing buffer maintains the RNA molecules in a denatured state, during electrophoresis, for at least 3 hours (2,3). The use of native agarose gels eliminates problems associated with toxic chemicals and the difficulties encountered when staining and blotting formaldehyde gels.

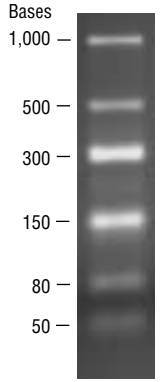
Sample Preparation: Two methods are recommended for preparing denatured RNA samples to be run on a native gel:

Method 1:
This method utilizes the 2X ssRNA Ladder Loading Buffer provided, and samples should be run on a native gel prepared with 1X TBE. This method **does not** always denature RNA molecules completely.

1. Combine on ice:

Low Range ssRNA Ladder (500 µg/ml):	2 µl (1 µg)
H_2O (RNase-free):	3 µl
2X RNA Ladder Loading Buffer:	5 µl
Total Volume	10 µl

2. Heat at 65°C for 5 minutes, chill on ice, load entire sample on gel.



1 µg of Low Range ssRNA Ladder was heated at 65°C for 5 minutes in 1X ssRNA Ladder Loading Buffer and visualized by ethidium bromide staining (2.0% TBE agarose gel).

Method 2:
This method utilizes formamide and formaldehyde in the sample as denaturants, and samples should be run on a native gel prepared with 1X MOPS buffer. This method is the most effective for denaturing RNA and should be used for precise sizing. The more denatured the RNA, the lower its affinity for EtBr, therefore this method requires 2–3 µg of ladder to be loaded to give good band intensity.

(see other side)

CERTIFICATE OF ANALYSIS

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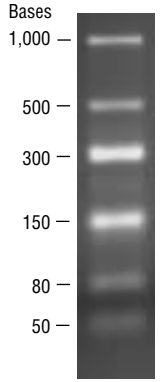

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CERTIFICATE OF ANALYSIS

1. Combine on ice:

Low Range ssRNA Ladder (500 µg/ml):	4–6 µl (2–3 µg)
H_2O:	up to 6 µl
10X MOPS:	2 µl
Deionized Formamide:	10 µl
Formaldehyde (37%)	2 µl
Total Volume	20 µl

2. Heat at 70°C for 5 minutes, chill on ice. Add 2 µl of 6X loading dye with bromophenol blue, load entire sample.

Method 3:

This method utilizes the 2X ssRNA Ladder Loading Buffer provided and samples should be run on a denaturing polyacrylamide-urea gel prepared with 1X TBE.

1. Combine on ice:

Low Range ssRNA Ladder (500 µg/ml):	0.5 µl (0.25 µg)
H_2O (RNase-free):	4.5 µl
2X ssRNA Ladder Loading Buffer:	5 µl
Total Volume	10 µl

2. Heat at 65°C for 5 minutes, chill on ice, load entire sample on gel.
3. Run gel according to manufacturer's conditions. All six bands of the marker can be resolved on a 6% gel. 0.5 µg/ml ethidium bromide will effectively stain the bands after electrophoresis.

Notes on Use: To avoid ribonuclease contamination: wear gloves, use RNase-free water for gels and buffers, wash equipment with detergent and rinse thoroughly with RNase-free water.

It is best to use freshly poured gels that are as thin as possible (i.e. 2–10 mm). Excessively long run times or high voltage can cause degradation of the bands on the gel. We recommend 4–8 volts/cm and running the bromophenol blue approximately 5 cm into the gel for good resolution.

Adding ethidium bromide to agarose gels and running buffer at a final concentration of 0.5 µg/ml will effectively stain the bands during electrophoresis

References:

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning*: *A Laboratory Manual*, (2nd ed.), (pp. 7.43–7.45). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. Liu, Y-C. and Chou, Y-C. (1990) *Biotechniques* 9, 558.
3. Sandra Cook, and Christina Marchetti, unpublished observations.
4. Dong Ma, and John Greci, unpublished observations.

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2X ssRNA Ladder Loading Buffer:	5 µl
Total Volume	10 µl

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