

# EUROMEDEX

## CERTIFICATE OF ANALYSIS

### Fast Ultra Low Range DNA Ladder ready-to-use

**#01B-0113**      **2x 500 µl**  
(for 50-333 applications)

**Lot**      Exp. date:

Supplied with 1ml 6X Orange Loading Dye Solution

**Store at room temperature** (or at +4°C for periods up to 6 months. For longer periods store at -20°C).

*In total 3 vials.*

#### Description

DNA Ladder, Ultra Low Range, ready-to-use, is designed for the fast sizing and quantification of DNA fragments both in the high throughput (46-96-well) gels and in the conventional agarose gels.

The Ladder bands are easily separated in the 10-20mm distance after 8-14 min electrophoresis on a high percentage agarose gels.

The Ladder is a mixture of five blunt-ended chromatography - purified individual DNA fragments (in base pairs): 200, 100, 50, 20, 10. These DNA fragments are dephosphorylated. They are suitable for the forward 5'-end labeling with T4 Polynucleotide Kinase (09-10311) (see Protocol on the back page).

The Ladder is ready-to-use – it is premixed with the 6X Orange Loading Dye Solution for direct loading on gels.

#### Storage and Loading Buffer

10mM Tris-HCl (pH 7.6), 10mM EDTA, 0.025% orange G, 0.005% xylene cyanol FF and 10% glycerol.

#### 6X Orange Loading Dye Solution

10mM Tris-HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60% glycerol and 60mM EDTA.

#### Protocol for Loading

**Step 1:** Mix gently

**Step 2:** Load 1µl per 1mm gel lane

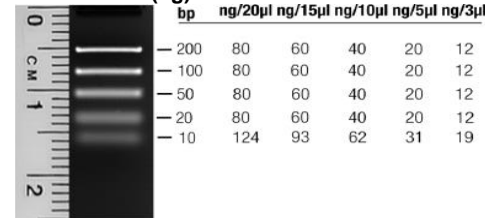
#### Recommendations

- Do not heat before loading.
- For sizing: Dilute your DNA sample with the 6X Orange Loading Dye Solution (10-0211, supplied with the Ladder): mix 1 volume of the dye with 5 volumes of the DNA sample. Load equal volumes of the DNA sample and the DNA Ladder.
- For quantification: Adjust the concentration of the sample to equalize it approximately with the amount of DNA in the nearest band of the Ladder.
- Visualize DNA by staining with ethidium bromide or with SYBR® Green I.
- Use TBE buffer both to prepare the gel and to run electrophoresis.

#### Quality Control Assay Data

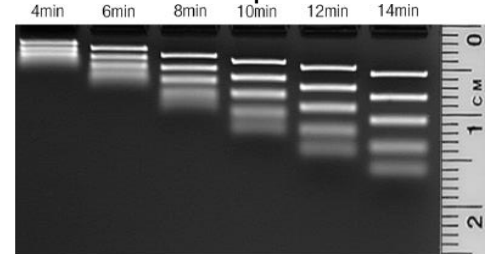
Well-defined bands are formed during agarose gel electrophoresis. The absence of nucleases is confirmed by a direct nuclease activity assay.

#### DNA amount (ng) in each band\*.



20µl/lane, 4.0% TopVision LE GQ Agarose (#R0491), 1X TBE, 7V/cm, 14min.

#### Time course of band separation\*.



20µl/lane, 4% TopVision LE GQ Agarose (#R0491), 1X TBE, 7V/cm.

\* Formation of diffused bands of small DNA fragments is a feature of agarose gel electrophoresis.

For **PROTOCOL for RADIOACTIVE LABELING** see back page

**Protocol for Forward Radioactive 5'-Termini Labeling Using T4 Polynucleotide Kinase (09-10311)**

1. Prepare the following reaction mixture:

- DNA Ladder Fast,

Ultra Low Range, ready-to-use: 2µl

- 10X Reaction Buffer A

(supplied with the enzyme): 2µl

- [ $\gamma$ -<sup>32</sup>P] or [ $\gamma$ -<sup>33</sup>P]-ATP (3.3pmol/µl): 2µl

- Water, nuclease-free (08-0412): 13µl

- T4 Polynucleotide Kinase (10u): 1µl

2. Incubate at 37°C for 30 minutes.

3. Add 1µl 0.5M EDTA, pH 8, and extract with an equal volume of chloroform.

4. Purify the labeled DNA on Sephadex™ G-50 column.

**Product Use Limitation.**

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

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