

Preparation of DNA Samples from Enzymatic Reaction Mixtures or with Samples Containing High Amounts of DNA Binding Proteins

This protocol is for the Preparation of DNA Samples from Enzymatic Reaction Mixtures or with Samples Containing High Amounts of DNA Binding Proteins.

Use Thermo Scientific 6x DNA Loading Dye & SDS (Cat #R1151) Solution to prevent the appearance of additional bands or gel shifts when analyzing:

- Probes after DNA restriction digestions, ligation or dephosphorylation reactions,
- DNA samples with high amounts of DNA binding proteins,
- DNA molecules with cohesive ends,
- or to stop an enzymatic reaction during kinetic experiments.

Use 6x DNA Loading Dye & SDS Solution to prevent the appearance of additional bands or gel shifts when analyzing:

1. Add 1 volume of 6x DNA Loading Dye & SDS Solution to 5 volumes of DNA sample.
2. Mix well.
3. Heat at 65 °C for 10 minutes.
4. Chill on ice, spin down and load.

Note:

The prepared sample can be stored at -20 °C and reused for electrophoresis after several freeze-thaw cycles.

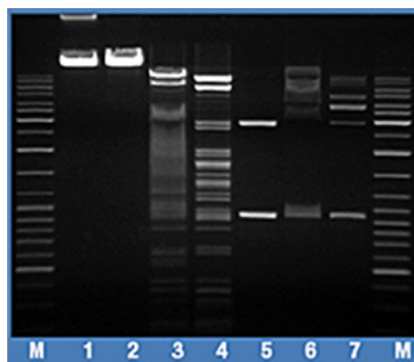


Figure. The effect of SDS on electrophoresis of DNA samples containing high amounts of DNA binding proteins.

M – Thermo Scientific GeneRuler™ DNA Ladder Mix

1 – 0.5 µg lambda DNA prepared for loading with Thermo Scientific 6x DNA Loading Dye

2 – 0.5 µg lambda DNA prepared for loading with 6x DNA Loading Dye & SDS Solution

3 – 0.5 µg lambda DNA digested with TsoI, probe prepared for loading with 6x DNA Loading Dye

4 – 0.5 µg lambda DNA digested with TsoI, probe prepared for loading with 6x DNA Loading Dye & SDS Solution

5 – 0.4 µg of the 2 fragment ligation mixture prior the addition of T₄ DNA Ligase

6 – 0.4 µg of the 2 fragment ligation mixture after the ligation with T₄ DNA Ligase, probe prepared for loading with 6x DNA Loading Dye

7 – 0.4 µg of the 2 fragment ligation mixture after the ligation with T₄ DNA Ligase, probe prepared for loading with 6x DNA Loading Dye & SDS Solution

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