OptiPrep™ Application Sheet M06

Isolation of plasmid DNA in self-generated gradients of iodixanol using DAPI as a fluorescent marker

- **OptiPrep™** is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **To access other Application Sheets** referred to in the text: return to the **2020Macroapp** file and select the appropriate M-number.

1. Background

Centrifugation in ethidium bromide (EtBr)-containing CsCl gradients is one of the standard methods of plasmid DNA isolation. The technique however suffers from a number of problems: difficulties in removing CsCl before further processing (ethanol extraction can lead to precipitation of CsCl) and the hazardous nature of EtBr (it intercalates DNA and is thus a potent mutagen). In addition, the density of CsCl gradients at the r_{max} may be close to the maximum permissible value for some rotors; this may overstress the rotor and potentially be catastrophic.

The technique described in this Application Sheet uses iodixanol and the fluorescent marker DAPI (4,6-diamidino-2-phenylindole). As iodixanol is non-ionic, plasmid DNA from iodixanol gradients can be analyzed by electrophoresis directly. Iodixanol is not precipitated by propan-2-ol or ethanol, nor does it inhibit restriction nucleases. Densities of nucleic acids in iodixanol (approx 1.10 g/ml) are much lower than in CsCl: the gradients required are thus of a much lower density and cannot stress rotors. Also because DAPI binds in the groove of the DNA it is very much less hazardous than EtBr.

2. Solutions required

A. 50 mM glucose, 25 mM Tris-HCl, pH 8.0, 10 mM EDTA.
B. Lysozyme
C. 0.1M NaOH, 1% (w/v) SDS (fresh)
D. 3 M sodium acetate, pH 4.8
E. Propan-2-ol (isopropanol)
F. 70% (v/v) ethanol
G. 1 mM EDTA, 10 mM Tris-HCl, pH 7.5
H. OptiPrep™
I. 0.5% (w/v) DAPI

Keep the following stock solutions at 4°C:
1 M Tris (free base), 12.1 g per 100 ml
100 mM $EDTANa_2 \bullet 2H_2O$ 3.72 g per 100 ml

Solution A: Dissolve 0.9 g glucose in 50 ml water, add 2.5 ml and 10 ml of Tris and EDTA stock solutions respectively; adjust to pH 8.0 with 1 M HCl; make up to 100 ml.

Solution D: Dissolve 40.8 g $NaOAc \bullet 3H_2O$ in 100 ml water. Dilute 10 ml glacial acetic acid to 58 ml with water (CARE!!). Mix 59 ml of NaOAc with 41 ml of HOAc. Check pH is 4.8.

Solution G: Add 1 ml each of Tris and EDTA stock solutions to 50 ml water; adjust to pH 7.5 with 1 M HCl; make up to 100 ml.

3. Protocol

3a. Preparation of crude plasmid DNA

1. Pellet the bacteria by centrifugation at 2000 *g* for 20 min.
2. Resuspend the pellet in 10 ml of Solution A and add lysozyme to a final concentration of 5 mg/ml.
3. Incubate the suspension at room temperature for 10 min, then add 20 ml of Solution C; gently mix and leave to incubate for 10 min at room temperature.
4. Place the mixture in ice and add 15 ml of Solution D and incubate for 10 min.
5. Centrifuge at 25,000 *g* for 30 min and carefully remove the plasmid-containing supernatant.
6. Add 0.6 vols of propan-2-ol; mix well and store at -20°C for at least 30 min.
7. Collect the precipitated DNA by centrifugation at 20,000 *g* for 30 min; wash the pellet in Solution F and finally dissolve in 10 ml of Solution G.

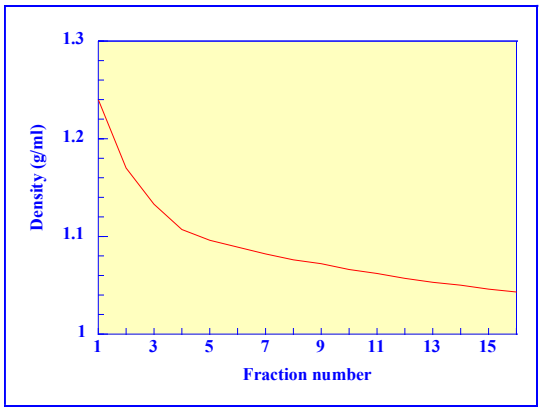

Figure 2 Density profile required for plasmid DNA fractionation.

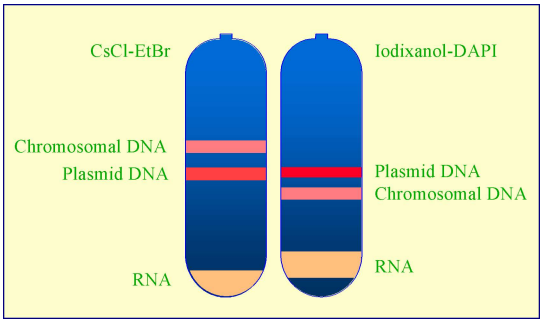

Figure 1: Banding of plasmid and chromosomal DNA in CsCl-EtBr and in iodixanol-DAPI gradients

3b. Purification of plasmid DNA

1. To the plasmid solution add OptiPrep™ to a final concentration of 27% (w/v) iodixanol and DAPI to 0.005%.

2. Transfer the solution to a sealed tube for a suitable near-vertical or low-angle fixed angle rotor. The example given in this Application Sheet is with a low-angle fixed-angle rotor (tube size 5 ml) centrifuged at 350,000g for 12-15 h at 5°C. The density profile of the gradient which is generated is shown in Figure 1 (see Note 1).

3. Observe the result with a UV illuminator and remove the plasmid DNA band using a hypodermic syringe (see Figure 2 and Note 2).

4. Analysis

Samples of plasmid DNA removed from the iodixanol gradients can be electrophoresed either directly or after digestion with the appropriate restriction nuclease, on an agarose gel. Agarose gel profiles show that the purity of the plasmid DNA from both EtBr-CsCl and the DAPI-iodixanol gradient was very similar. Samples from the CsCl gradient need to be desalted either by ethanol precipitation after dilution or by passage over a spin column before electrophoresis; the smearing of the plasmid DNA observed in the CsCl purified sample is due to residual CsCl in the sample (see Note 3).

5. Notes

1. Other rotors with a shorter sedimentation path length may require significantly less time to self-generate the gradient and band the DNA. **For more information about self-generated gradients see Application Sheet M03**.
2. In the familiar EtBr-CsCl gradients the plasmid DNA bands denser than the denatured linear chromosomal DNA, because it binds less EtBr (Figure 2). In DAPI-iodixanol gradients the band of native plasmid DNA will be visible as a bright, light blue band, while the denatured chromosomal DNA is observed as a fainter band beneath (Figure 2).
3. More information about the isolation of nucleic acids in iodinated density gradient media can be obtained from refs 1 and 2.

6. References

1. Rickwood, D. (1992) *Centrifugal methods for characterizing macromolecules and their interactions* In Preparative Centrifugation - A Practical Approach (ed D. Rickwood) IRL Press at Oxford University Press, Oxford, UK, pp. 143-186
2. Rickwood, D. and Patel, N. V. (1996) *An improved method for the isolation of plasmid DNA using OptiPrep-DAPI gradients* Mol. Biol. Cell, **7**, 162a

serumwerk bernburg
Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwwerk.com • serumwerk.de