OptiPrep™ Application Sheet C39

Purification of bacteria from soil, clinical specimens and food

- 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 Cell Index; key Ctrl "F" and type the C-Number in the Find Box.
- Section 7 briefly addresses the isolation of bacteria from host tissues/organisms
- Note that purification of obligate intracellular bacteria is addressed in **Application Sheet C53**

1. Background

The use of a Nycodenz® density barrier of approx 1.3 g/ml density to isolate bacteria from soil and surface water samples was first reported in 1995 by Lindahl and Bakken [1]; this paper also contains a review of the methods used for soil dispersion. The preferred method for the detachment of the bacteria from the soil samples is the use of a rotating blades blender, normally a Waring blender. Other techniques have been investigated including sonication, reciprocal shaking and a rotating pestle, but the blender was preferred over these other techniques as the most reliable [1]. The sterile medium used to suspend the soil particles is quite variable; in some cases water is used; other examples are a 0.05 M phosphate buffer, 0.15% NaCl, a phosphate-buffered saline solution, organic buffer such as 10-50 mM Tris-HCl, pH 7.5-8.0, which may be supplemented with either 0.2M NaCl or 1 mM EDTA.

The published literature widely reports the use of this Nycodenz® 1.3 g/ml density barrier. In terms of the recovery, purity and functional integrity of the bacteria, Nycodenz® has been shown to be superior both to inorganic salts such as CsCl and Na_2WO_4 and other non-ionic gradient media such as metrizamide and Percoll® [2,3]. Lindahl [4] noted that the Nycodenz® barrier method could effect an almost complete separation of bacteria from the soil particles and percentage recoveries can be as high as 80% [5]. There are a few examples of the use of a slightly lower density for the barrier, e.g. 1.26 g/ml [6].

The same Nycodenz® density barrier strategies have been used for the isolation of bacteria from fecal matter [7,8] and from food [9]. Hazebrouck et al [8] used a 1.26 g/ml barrier.

Now the methodology for the isolation of *Salmonella enterica* from soil has been adapted to iodixanol [10] and the latter's availability as a sterile solution of density 1.32 g/ml (OptiPrep™) makes it very easy to use in such an application. Neat OptiPrep™ is used directly as the density barrier or diluted with a buffered isoosmotic solution, i.e. there is no need to for the lengthy preparation of a dense solution from Nycodenz® powder nor is sterilization of the solution required. Because of the ease of solution preparation the method given in Sections 3-5 is based solely on the use of OptiPrep™.

In a recent paper by Pascaud et al [11], a three-layered gradient of 1.10, 1.15 and 1.30 g/ml (2 ml of each) was produced by dilution of OptiPrep ™ with 0.25 M sucrose, 6 mM EDTA, 60 mM Tris-HCl, pH 7.4 and the soil suspension (5 ml) layered on top. Nousiainen et al [12] have also adapted the standard Nycodenz® to OptiPrep™.

2. Centrifugation strategies

In the original methodology [1], 2 ml of the suspension was layered upon 7 ml of the Nycodenz® barrier and centrifuged at 10,000 *g* for 20 min. The volume ratio of sample:barrier is normally 2-3:1. Much larger scale separations are also possible e.g. 25 ml over 11.6 ml [13] 90 ml of sample over 30 ml of density barrier [14]. The most frequently used centrifugation conditions are 10,000 *g* for 20-30 min; occasionally higher *g*-forces are used, e.g. 25,000 *g* for 1 h [15] occasionally much lower, e.g. 3000 *g* for 20 min with a sample:barrier volume ratio of 10 [16].

The methodology has also been adapted for multiple analyses to the use of approx 2 ml micro-centrifuge tubes with 800 µl layered over 700 µl of density barrier [e.g. ref 6]. Ref. 15 describes the use of a 1.34 g/ml barrier.

3. Reagents required

A. OptiPrep™ (shake the bottle gently before use)
B. Saline (sterile) or other suitable buffer (see Section 1)

4. Centrifuge requirement

High-speed centrifuge or ultracentrifuge with swinging-bucket rotor of appropriate tube volume, or microcentrifuge

5. Protocol

1. Sieve the soil through a 2 mm mesh and suspend in saline (10 g soil per 100 ml).

2. Macerate in a Waring blender (or other rotating blades device) at full speed for a total time of 3-5 min at 4°C, using 1 min "bursts" and 1 min "rests" to dissipate any heat (see Note 1).

3. *Density barrier method:* Either use pure OptiPrep ™ for the barrier or mix 10 vol. of OptiPrep ™ with 0.1 vol. of a 100x buffer (see Notes 2). Then layer the macerated sample over the chosen density barrier using a volume ratio of 4:2.5 (see Notes 3 and 4).

4. *Discontinuous gradient method:* Dilute OptiPrep ™ with 0.25 M sucrose, 6 mM EDTA, 60 mM Tris-HCl, pH 7.4 to produce 14%, 25% and 55% (w/v) iodixanol. Layer 2 ml of each in a tube and layer the soil suspension (5 ml) layered on top (see Note 5). For more information on production of gradient solutions using a sucrose solution **see Application Sheet S01.**

5. *Density barrier method:* Centrifuge at 10,000-25,000 *g* at 4°C for 20-40 min (see Note 6) and then harvest all of the material above the soil pellet (see Note 7).

6. *Discontinuous gradient method:* Centrifuge at 2600 *g* for 1 h; then aspirate the liquid above the 55% (w/v) iodixanol layer.

7. Dilute the bacterial suspension with 3 vol. of sterile saline; pellet the bacteria at 10,000-20,000 *g* for 20-60 min and resuspend in a suitable medium (see Note 8).

6. Notes

1. The precise conditions for dispersion are quite variable. Some are much more gentle, e.g. 3x1 min at low speed, in others the rest periods of much longer duration are used, e.g. 5 min. If a speed is stipulated it is often approx. 20,000 rpm.
2. If the presence of a buffer and/or saline is required in the density barrier, this dilution step will only cause a small change in the density of the 60% (w/v) iodixanol.
3. The relative volumes of sample and barrier are also variable, but usually the sample volume exceeds that of the barrier and ratio given is one that is commonly used. Rapp et al [17] underlayered 15 ml of the processed soil suspension with 9 ml of OptiPrep™.
4. In a recent paper by Pascaud et al [18] the density of the barrier was reduced to 1.20 g/ml, i.e. approx. 36% (w/v) iodixanol (OptiPrep™ diluted with a buffered solution of 0.25 M sucrose containing 6 mM EDTA).
5. Pascaud et al [18] used a three-layer gradient of 1.10, 1.15 and 1.30 g/ml; this is equivalent to approx. 14.5, 25 and 55% (w/v) iodixanol when OptiPrep™ is diluted with a buffered solution of 0.25 M sucrose. After centrifugation at 2,600 *g* for 1 h, the bacteria banded on top of the 1.15 g/ml layer.
6. The time for the centrifugation will vary with the total volume of sample + density barrier; for larger volumes the time may need to be increased.

7. Often the bacteria are harvested from the interfacial material and only the top half of the cushion. Because of the high density of the barrier the harvest requires considerable dilution (maybe as much as 10x) with buffer in order to reduce the density of the collected liquid.
8. The centrifugation conditions used to pellet the bacteria will depend on the sedimentation properties of the bacteria, the amount of cushion in the harvest and the total volume of the bacterial suspension after dilution; they vary from 16,000 *g* for 60 min to 100-200,000 *g* for 10-20 min.

7. Isolation of bacteria from a host tissue or organism

Because of the great variability in host tissue or organism, only a brief outline of a possible strategy for the study of the bacteria consortium. Woyke et al [19] studied the bacterial populations in annelids by loading an extract in PBS on to a 5 ml 1.083-1.146 g/ml Nycodenz® (equivalent to 15-27.5% w/v) gradient. It was centrifuged at 10,000 *g* for 1 h and collected in approx 0.25 ml fractions. Bacteria were harvested from the densest fractions and the metagenomic high MWt DNA analyzed. As far as is known, no similar separations have been carried out using OptiPrep™, although it is highly likely that gradients covering a similar density range would be effective.

8. References

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