# OptiPrep™ Application Sheet M07

## Analysis of mammalian and non-mammalian HDL, LDL and VLDL

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Mini-Review "Fractionation of plasma lipoproteins" (MM01)** provides a comprehensive up-to-date bibliography of all the published papers reporting the use of OptiPrep™: to access **MM01** return to the initial list of Folders and select "**Mini-Reviews**"
- To access other Application Sheets referred to in the text return to the Macromolecules and Macromolecular Complex Index; key Ctrl "F" and type the M-Number in the Find Box

### 1. Background

Although centrifugation is the "gold standard" method for the fractionation of plasma lipoproteins, sequential flotation by incrementally increasing the density of the plasma with KBr is tedious (approx. 3 days) and the use of KBr/NaCl gradients is technically difficult. In addition, for many of the subsequent analytical techniques or for further processing it is often necessary to remove the salt by dialysis, often adding a further 12 h to the procedure. Moreover, the use of high salt concentrations may cause the removal of surface apolipoproteins from lipoproteins.

Self-generated gradients iodixanol in vertical or near-vertical rotors (3-12 ml tubes) considerably simplify the separation procedure [1,2]. After removal of chylomicrons, plasma is adjusted to a suitable starting density by addition of a small volume of OptiPrep; loaded into a centrifuge tube and centrifuged for 2-3 h. The procedure is summarized in the flow chart that is Figure 1.

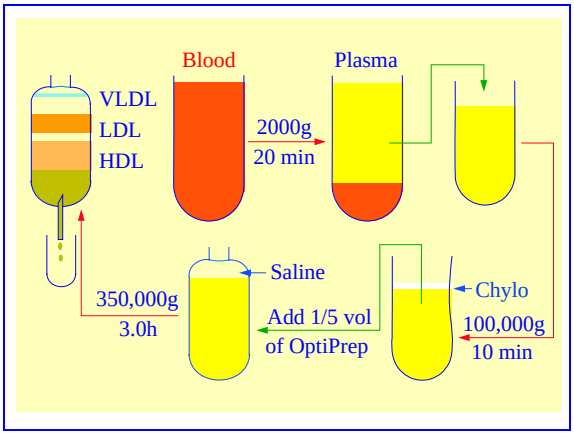


**Figure 1** Flow chart: fractionation of lipoproteins from human plasma

- This Application Sheet describes the basic technique. For some of the possible variations in gradient conditions, which may be more appropriate to subfractionation of lipoprotein classes, see **Application Sheet M08**.

There are some significant advantages to the use of self-generated gradients over the more traditional sequential flotation methods

- Ease of sample handling
- Shorter separation times reduce possible cholesterol oxidation

### 2. Solutions required

**A.** OptiPrep™

**B.** Hepes-buffered saline: 0.85% (w/v) NaCl, 10 mM Hepes-NaOH, pH 7.4

Keep Hepes (free acid) as 100 mM stock solution at 4°C:
2.38 g Hepes per 100 ml water

Solution B: Dissolve 0.85 g NaCl in 50 ml water, add 10 ml of buffer stock solution; adjust to pH 7.4 with 1 M NaOH; make up to 100 ml

### 3. Ultracentrifuge rotor requirements

Any vertical, near-vertical or small volume fixed angle rotor (sedimentation path length of approx 17 mm or less) for an ultra- or microultra- centrifuge. Tube volumes of 2-12 ml are normally suitable. Most studies have been carried out using the following Beckman rotors: TLN100 near-vertical, VTi65.1 vertical, NVT65 near-vertical and NVT65.2 near-vertical (see Note 1) but others such as the TLV-100, and NVT90 will give similar separations. Sorvall ultracentrifuge vertical rotors such as the TV865, TV1665, Stepsaver™ 65V13, Stepsaver™ 70V6 and microultracentrifuge rotors such as the S120-VT and RP100-VT are alternatives.

## 4. Protocol (for 3.3 ml tubes in the Beckman TLN100 near-vertical rotor)

1. Using freshly drawn blood (1 mM EDTA as anti-coagulant), pellet the cells at 2000 *g* for 15 min (see Notes 2 and 3).

2. Remove chylomicrons from the plasma by centrifugation at 100,000 *g* for 10 min (see Note 4).

3. Mix 4 vol of plasma with 1 vol of OptiPrep™ (12% iodixanol final concentration) and transfer 2.8 ml to an OptiSeal™ tube (see Note 5).

4. Layer Solution B on top to fill the tube (see Notes 6 and 7).

5. After sealing the tube, centrifuge at approx 350,000 $g_{av}$ for 2.5-3 h at 16°C, using slow acceleration to and deceleration from 2000 rpm.

6. Collect the gradient in 0.1 - 0.2 ml fractions by tube puncture and analyze the fractions (see Notes 8-13).

## 5. Notes

1. The gradient density profiles obtained after 2-3 h in the TLN100 near vertical rotor (Figure 2) are the most useful for the majority of lipoprotein separations, although the S-shaped profiles (obtained after 1 h) containing a shallow median section might be adapted to lipoprotein subfractionation, so long as this is sufficient time for the lipoproteins to reach their banding density. For more information on the formation of self-generated gradients see **Application Sheet M03.**
2. Citrate may also be used as anticoagulant; heparin has not been tested. Serum may be used instead of plasma.
3. For species other than human go to the Axis-Shield Abstract Database (see Section 8).
4. The conditions used to float chylomicrons vary widely, the higher g-forces for shorter times used here are very effective.

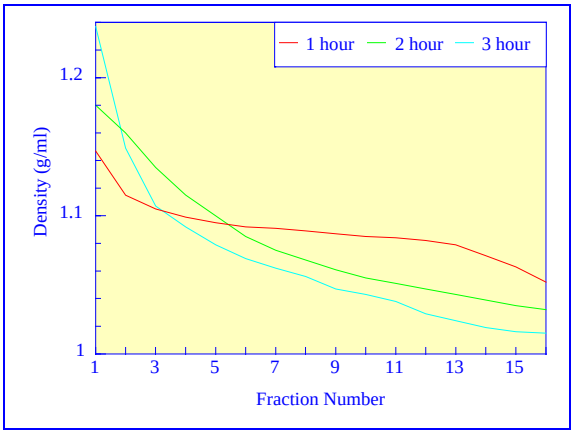


**Figure 2** Density profiles in TLN100: 12% iodixanol centrifuged at 350,000g at 16°C.

5. Other concentrations of iodixanol may be suited to the purification of specific lipoproteins and for plasma from other species.
6. The saline on top of the sample not only conveniently fills the tube; it minimizes the tendency of the VLDL to adhere to the wall of the tube. This is particularly important with vertical rotors. It also enhances the separation of the VLDL from the lightest LDL in all rotors.
7. In vertical rotors it may also be beneficial to include a small volume of 20% iodixanol (approx 0.5 ml) as a cushion to prevent soluble proteins sedimenting on to the outer side of the tube This is less important in near-vertical or fixed-angle rotors.
8. Tube puncture generally provides a better recovery of VLDL from an Optiseal tube than does upward displacement. On the other hand the plasma proteins at the bottom of the tube tend to contaminate the dense HDL fractions more seriously if tube puncture is used. For more information on harvesting gradients see **Application Sheet M04**.
9. Fractions from iodixanol gradients can be analyzed directly by agarose gel and SDS-PAGE electrophoresis and for cholesterol and triacylglycerol without dialysis of the medium. Iodixanol is non-ionic and does not interfere with any of these analytical procedures. A typical agarose gel is shown in Figure 3.

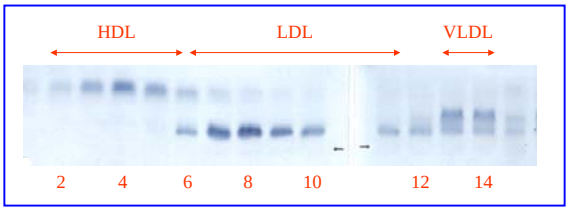


**Figure 3** Sudan black stained agarose gel electrophoresis profile of human plasma lipoproteins. Gradient was collected dense-end first (fraction 1).

10. The banding densities of LDL and particularly HDL, are lower than in salt gradients. In iodixanol gradients apolipoproteins retain any bound water. In hyperosmotic salt gradients this water is lost (see ref 2 for more details).
11. Not only is excellent resolution achieved of the major classes of lipoprotein, the method also produces a significant concentration of the lipoproteins. A typical agarose gel electrophoresis profile of a gradient is shown in Figure 3. From each fraction, 3 µl was applied directly to a commercial agarose gel and after electrophoresis was stained in Sudan black.
12. **Application Sheet M08** describes some of the modifications to the gradient system that can be used in analysis of the sub-classes of lipoproteins.
13. If the separation is carried out in a tube for the VTi65.1 vertical rotor, the tall format of this tube promotes the maintenance of the resolving power of the gradient during unloading.

♦ There are a number of published reviews on the gradient technology used for plasma lipoprotein fractionation and these are listed below in Section 6 [3-6]. Some of these also provide an overview of other methodologies and ref 5 describes the adaptation of the methodology described above to the use of larger volumes of plasma.

## 6. References

1. Graham, J.M., Higgins, J.A. and Gillot, T. (1995) *A new method for the rapid separation of plasma lipoproteins* Atherosclerosis, **115 (Suppl)**, S123
2. Graham, J., Higgins, J. A., Gillott, T., Taylor, T., Wilkinson, J., Ford, T. and Billington, D. (1996) *A novel method for the rapid separation of plasma lipoproteins using self-generated gradients of iodixanol* Atherosclerosis, **124**, 125-135
3. Patterson, B. W. (2002) *Methods for measuring lipid metabolism in vivo* Curr. Opin. Clin. Nutr. Metab. Care, **5**, 475-479
4. Langlois, M.R. and Blaton, V.H. (2006) *Historical milestones in measurement of HDL-cholesterol: Impact on clinical and laboratory practice* Clin. Chim. Acta, 369, 168-178
5. Billington, D., Maxwell, E., Graham, J.M. and Newland, P. (2007) *Large-scale preparation of human low- and high-density lipoproteins by density gradient centrifugation using iodixanol* Anal. Biochem., **367**, 137-139
6. Yee, M.S., Pavitt, D.V., Tan, T., Venkatesan, S., Godsland, I.F., Richmond, W. and Johnston, D.G. (2008) *Lipoprotein separation in a novel iodixanol density gradient, for composition, density and phenotype analysis* J. Lipid Res., **49**, 1364-1371



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