# OptiPrep™ Application Sheet V20

## Purification of Group IV ((+)ss) RNA viruses: *Flaviviridae* family: hepatitis C particles in a self-generated gradient

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The Axis-Shield Mini-Review (MV06) "Purification and analysis of hepatitis C virus" summarizes the published methods and provides a full bibliography of all published papers reporting the use of iodixanol gradients for their purification; to access return to the initial list of Folders and select "**Mini-Reviews**".
- For the purification of the virus from cultured cells in pre-formed gradients by buoyant density or sedimentation velocity see Application Sheet V19.
- To access other Application Sheets referred to in the text return to the Virus Index; key Ctrl "F" and type the V-Number in the Find Box.

### 1. Introduction

The methodology described in this Application Sheet was primarily developed by the groups based in the Institutes of Cellular Medicine and of Cell and Molecular Biosciences at the University of Newcastle (UK) for the recovery of hepatitis C virus particles from macerates of human infected liver, removed during transplantation and processed directly or from frozen material. The macerated samples have been analyzed in a self-generated iodixanol gradient [1,2]. Although the use of a self-generated gradient is not a common approach for hepatitis C virus, the method is used widely for many retroviruses and has the major advantage of simplicity and safety of sample handling, especially when human samples are involved.

- The methodology has now been extended to the use of plasma samples and also for studies on the virus from cultured cells. See Section 6 for more information on this.

### 2. Solutions required

**A.** OptiPrep™

**B.** Buffer: 0.25 M sucrose, 15 mM EDTA 30 mM Tris-HCl, pH 8.0

**C.** Tisssue lysis buffer: 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.2% bovine serum albumin (BSA)

Add protease inhibitors to Solution C as required.

Keep the following stock solutions at 4°C:
200 mM Tris (free base): 2.42 g per 100 ml water
100 mM EDTA ($Na_2$•$2H_2O$): 3.72 g per 100 ml water
1 M NaCl: 5.85 g per 100 ml

Solution B: Dissolve 8.5 g sucrose in 50 ml water; add 15 ml each of Tris and EDTA stocks; adjust to pH 8.0 with 1 M HCl and make up to 100 ml

Solution C: To 40 ml water add 15 ml and 25 ml of NaCl and Tris stocks respectively; adjust to pH 7.4 with 1 M HCl; dissolve 0. 2 g of BSA; check pH and make up to 100ml. Make up fresh.

### 3. Rotor requirements

For gradient purification: fixed-angle rotor with 10 ml sealed tubes (e.g. Beckman 50Ti) or a vertical or near-vertical rotor (e.g. Beckman VTi65.1 or NVT65) with similar tubes (see Section 5)

### 4. Protocol

1. Homogenize 400-500 mg liver in 10 ml of Solution C using up to 60 strokes of the pestle of a tight-fitting Dounce homogenizer. This must be carried out in an isolation cabinet to avoid exposure to aerosols.

2. Centrifuge at 8000 *g* for 5 min at 4°C, then pass the supernatant through a 0.45 µm filter.

3. In tubes for the chosen rotor mix 4.2 ml of OptiPrep™, 3.3 ml of Solution B and 2.5 ml of the supernatant (see Section 5, Notes 1 and 2).

4. In a 10 ml fixed-angle tube centrifuge at 165,000 $g_{av}$ for 24 h at 4°C (see Section 5, Note 2).

5. Allow the rotor to decelerate using a slow-deceleration program below 2000 rpm, or turn off the brake below 2000 rpm, then collect the gradient in 0.5-0.7 ml fractions, low density end first. For more information on harvesting gradients see Application Sheet V04.

**5. Notes**

1. Note that this recipe for the self-generated gradient is a slightly simplified version of that in ref. 1. In that example 2.2 ml of the macerate was mixed with 7.8 ml of a solution containing 32% (w/v) iodixanol, 0.1 M sucrose, 13 mM Tris-HCl, pH 8.0 and 6.5 mM EDTA [1]. In a later example [2] 4.2 ml of the macerate was mixed with 6.24 ml of a solution containing 40% (w/v) iodixanol, 60 mM sucrose, 16 mM Tris-HCl, pH 8.0 and 27 mM EDTA [1]. The final concentration of iodixanol in both cases was however the same at approx. 25% (w/v).
2. Nielsen et al [1,2] used a Beckman 50Ti fixed-angle rotor with approx. 10 ml tubes. Self-generated gradients are more usually prepared in high-performance short sedimentation path length vertical and near-vertical rotors. If these rotors are used at 265-365,000 *g* self-generated gradients can be formed in 1-3 h see for example the isolation of Herpes virus in Application Sheet V08. It should be noted however that retention of viral infectivity might be enhanced at lower g-forces.

**6. Other sample applications**

More recently the self-generated gradient approach has been extended to the analysis of human plasma samples. In these samples the particles of interest are primarily low density, hence the reduced iodixanol concentration. Felmlee et al [3] mixed 2 ml of plasma with 8 ml of a solution containing 16% (w/v) iodixanol, 2.5 mM EDTA, 12.5 mM Tris-HCl, pH 8.0, 176 mM sucrose and 6.25 mM of both $MgSO_4$ and $MgCl_2$. A higher performance Beckman 70Ti fixed-angle rotor was used at approx. 266,000 $g_{av}$ for 16 h. A smaller plasma volume (0.5 ml) was used by Bridge et al [4], adjusted to very similar concentrations of iodixanol, buffer etc. In the lower performance 50Ti rotor the time required at 165,000 $g_{av}$ was 24 h. Sheridan et al [5] used this latter method for plasma. Virus from cultured cells has also been analyzed in self-generated iodixanol gradients [6].

♦ For more information on the creation of self-generated gradients see Application Sheet V03

**7. References**


1. Nielsen, S.U., Bassendine, F., Burt, A.D., Bevitt, D.J. and Toms, G.L. (2004) *Characterization of the genome and structural proteins of hepatitis C virus resolved from infected human liver* J. Gen. Virol., **85**, 1497-1507
2. Nielsen, S.U., Bassendine, M.F., Martin, C., Lowther, D., Purcell, P.J., King, B.J., Neely, D., Toms, G.L. (2008) *Characterization of hepatitis C RNA-containing particles from human liver by density and size* J. Gen. Virol., **89**, 2507-2517
3. Felmlee, D.J., Sheridan, D.A., Bridge, S.H., Nielsen, S.U., Milne, R.W., Packard, C.J., Caslake, M.J., McLauchlan, J., Toms, G.L., Neely, R.D.G. and Bassendine, M.F. (2010) I*ntravascular transfer contributes to postprandial increase in numbers of very-low-density hepatitis C virus particles* Gastroenterology **139**, 1774–1783
4. Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Nielsen, S.U., Thomas, H.C., Taylor-Robinson, S.D., Neely, R.D.G., Toms, G.L. and Bassendine, M.F. (2011) *Insulin resistance and low-density apolipoprotein B-associated lipoviral particles in hepatitis C virus genotype 1 infection* Gut, **60**, 680-687
5. Sheridan, D.A., Bridge, S.H., Felmlee, D.J., Crossey, M.M.E., Thomas, H.C., Taylor-Robinson, S.D., Toms, G.L., Neely, R.D.G. and Bassendine, M.F. (2012) *Apolipoprotein-E and hepatitis C lipoviral particles in genotype 1 infection: Evidence for an association with interferon sensitivity* J. Hepatol., **57**, 32–38
6. Vassilaki, N., Friebe, P., Meuleman, P., Kallis, S., Kaul, A., Paranhos-Baccalà, G., Leroux-Roels, G., Mavromara, P. and Bartenschlager, R. (2008) *Role of the hepatitis C virus core+1 open reading frame and core cis-acting RNA elements in viral RNA translation and replication* J. Virol., **82**, 11503-11515