# Optiprep gradient preparation for virus separation

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## Abstract

The purpose of this protocol is to generate Optiprep gradients for isolating viruses

**Citation:** Ilana Gilg Optiprep gradient preparation for virus separation. **protocols.io** dx.doi.org/10.17504/protocols.io.f6dbra6
**Published:** 20 Oct 2016

## Materials

Ethyl alcohol, Pure 200 proof, for molecular biology E7023 by Sigma Aldrich

Optiprep ( Iodixanol) D1556-250ML by Sigma Aldrich

- 1.4 Ultraclear 13 ml Beckman Centrifuge Tubes 344059 by Contributed by users
- Ring stand with tube clamps by Contributed by users
- 15 ml sterile falcon tubes and rack by Contributed by users
- Parafilm by Contributed by users
- 20 ml luer lock syringe by Contributed by users
- 1.5 or 3 ml luer lock syringe by Contributed by users
- 20 gauge 0.5 inch needle with luer lock by Contributed by users
- Gloves by Contributed by users
- 5 ml serologicals and pipetman or 5 ml pipettor with sterile tips by Contributed by users
- Ultracentrifuge with swinging bucket rotor by Contributed by users
- F/2 medium MKK50L by Contributed by users

## Protocol

### Step 1.

### Optiprep preparation

UV and sterilize class II cabinet with reagent alcohol. Work with gloves and sleeve guards to prevent contaminating the hood.

### Step 2.

Label 5 15ml falcon tubes with 25%, 30%, 35%, 40% and 45% labels. Label a 50 ml falcon tube

"Optiprep". Remove OptiPrep solution from the fridge and wipe down sides, cap and septum with alcohol. Pierce septum with needle (20 gauge 1/2 inch needles work best) attached to appropriately-sized syringe and aseptically remove 15-16 ml of OptiPrep for every 2 gradients you intend to make. Place Optiprep in the sterile 50 ml falcon tube.

**Step 3.**

Make 25%, 30%, 35%, 40% and 45% Optiprep solutions in the labeled falcon tubes. You will need 2.5 ml of each solution for every gradient you plan to make. Mix well.

For example, if you are making 2 gradients, you will prepare 5 ml of each solution as follows:

25% = 3 ml media + 2 ml Optiprep

30% = 2.5 ml media + 2.5 ml OptiPrep

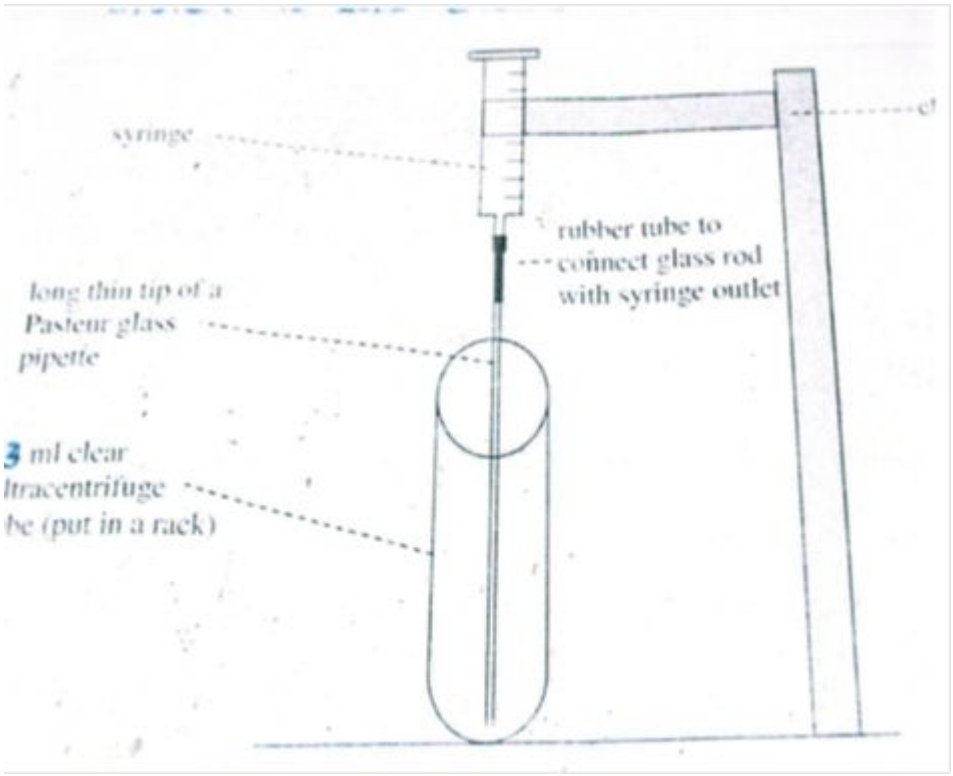
35% = 2.1 ml media + 2.9 ml OptiPrep

40% = 1.7 ml media + 3.3 ml OptiPrep

45% = 1.25 ml media + 3.75 ml OptiPrep

**Step 4.**

Make a loading system for the centrifuge tubes by attaching the thin end of a pasteur pipet to a 5 ml syringe with parafilm or a rubber tube. Hold the syringe with a ring clamp so that the end of the Pasteur pipet tip hovers just above the bottom of the tube.




**Step 5.**

Pipette 2.4 ml of 25% solution into the syringe. It will begin to flow into the centrifuge tube via gravity. When nearly all the solution has left the syringe, add 2.4 ml of the 30%. Repeat for all solutions.

**NOTE**: Try not to let the syringe run dry as it will introduce air bubbles into the tube and disrupting the reagent. If this happens, you can restart the flow by placing your thumb on the top of the syringe to generate and seal and then move your thumb up and down to increase the pressure in the syringe.

**Step 6.**

Once all tube has been poured (all percent solutions have been added), seal the top with parafilm and place the tubes in the fridge. Allow the tubes to sit overnight to form a continuous gradient.

**NOTE**:  Tubes may sit in the fridge for several weeks and will still be good as long as they are sealed.

**Step 7.**

**Separating viruses on the Optiprep gradient**

The next morning, pipette 0.5 - 4 ml of your viral concentrate onto each gradient. Balance the tubes using sterile growth medium if necessary (or whatever solution the virus sample is suspended in).

Centrifuge the tubes in a swinging bucket rotor in the Beckman Advanti J30-I (use rotor JS-25.15) at

max speed (40,000 rpm) for at least 4 hours at 8˚C.

**NOTE**: Occasionally you will need to spin for longer than 4 hours to achieve separation. In these cases an overnight or 24 hour spin may be required.

**Step 8.**

Remove the tubes carefully out of the rotor and place them on a standing clamp to visualize the bands.

**NOTE**:  Sometimes the bands are not visible until you suspend the tubes in a beaker of water, remove and back light them.

**Step 9.**

**Extracting bands**

Extract the bands separately using sterile needles and a 3 ml syringe.

**NOTE**: You must use 20 gauge half-inch needles at this stage to avoid puncturing both walls of the tube! Do not use any needles longer than ½".

- Wipe the sides of the tube wall with ethanol. Place the syringe just below the band and puncture the wall. Aspirate and remove the band with the syringe until the band disappears. It usually results in about 400 ul – 1 ml volume removed.
- Place a piece of scotch tape over the hole in the tube to prevent the gradient from leaking out.
- Place the sample into a 1.5 ml centrifuge tube and store at 4˚C until ready to use.

**NOTE**: If you have multiple bands, extract in the order from lowest to highest. This will prevent any leakage from the puncture site from possibly contaminating the wall next to your lower bands.

## Warnings

Take care when using the needle not to stick yourself. Also, before centrifuging, make sure that all tubes are perfectly balanced with a scale before loading them into the rotor.

Wear gloves and lab coat at all times during this procedure