

Protocol for Preparation of LDL from Human Plasma

1. Order two units of platelet-rich plasma from New York Blood Center (Tel: 212-468-2177; Fax: 212-468-2009; Contact person: Tommy)

Deadline of ordering is every Wednesday for the following week's order.

Plasma is prepared on the day of ordering. For example, place an order on a Monday, and the plasma would be prepared that day and ready for pick-up after 6:00 pm.

A copy of the order form appears at the end of this protocol.

2. Assume density of plasma to be 1.006. Adjust density to 1.019 with NaBr:

$$\text{___ g NaBr} = \frac{V_i (D_f - D_i)}{1 - (V' D_f)}$$

$V' = 0.24$ when $D_f = 1.019$

$V' = 0.244$ when $D_f = 1.063$

$V' = 0.246$ when $D_f = 1.09$

D_f = final concentration

D_i = initial concentration

3. Centrifuge at 45,000 rpm in a 50.2 Beckman centrifuge rotor for ~24 h at 10°C.

4. Carefully remove and discard the top lipid layer (VLDL). Use the densitometer to measure the density of the clear middle layer. Discard that layer and harvest the orange bottom layer of each tube and pool together (~30 ml per unit).

5. Adjust the density to 1.063 with NaBr and centrifuge as above for another 24 h.

6. Carefully harvest the top orange layer and pool. Check the density of the middle clear layer.

7. Adjust density to 1.09 with NaBr and centrifuge at 35,000 rpm in a 50.3 Beckman centrifuge rotor for ~16 h. Harvest the tops and keep as concentrated as possible (~ 1 ml per unit).

8. Dialyze multiple changes against LP buffer (150 mM NaCl, 1 mM EDTA PH 7.4) and then sterilize with 0.45- μ m Millipore syringe filter.

9. Assay an aliquot for protein concentration (Lowry) and store at 4°C under argon.

△ New York Blood Center

**Central Laboratory Facility
Component Laboratory
150 Amsterdam Avenue N.Y. N.Y. 10023**

Date: _____

Facility: _____

Doctors Name: _____

Phone Number: _____

Fax Number: _____

Contact Number: _____

Address: _____

P.O Number: _____

Fed. Ex. #: _____

DAY	DATE	AMT & PRODUCT*
MONDAY		
TUESDAY		
WEDNESDAY		
THURSDAY		
FRIDAY		

* PLEASE SPECIFY HOW MANY UNITS OF WHOLE BLOOD AND HOW MANY UNITS OF LUEKOCYTES IF BOTH ARE NEEDED.