



Lipoprotein separation by FPLC

Version: 1.0

Replaced by version: N/A

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Summary: *(This area will include the immutable area of the protocol. This is a brief description of the purpose and expectations of this protocol.)*

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
FPLC Buffer		See Reagent Preparation	
FPLC Equipment		See Protocol Below for details	
Eppendorf tubes	48		

Protocol:

1. Make up 5 liters of FPLC buffer (0.15 M NaCl, 0.01 M Na₂HPO₄, 0.1 mM EDTA, pH 7.5) and store at room temperature. The FPLC buffer should be degassed prior to use by adding 500 ml to a media bottle. Cover the bottle loosely and place it in an ultrasonic cleaner water bath for 30 minutes at room temperature.
2. The FPLC setup used in the Breslow lab is by Pharmacia:
Liquid Chromatography Controller LCC-500
Pump P-500
Single Path UV Monitor (optional)
Motor Valve MV-7
2 Superose6 FPLC columns in series (Pharmacia HR10/30 code # 17-0537-01)
Fraction Collector FRAC-100
3. Run the wash program and wash the system with 100 ml FPLC buffer at a flow rate of 0.3 ml/min

4. Place 48 Eppendorf tubes in the fraction collector.
5. Place 150 to 200 µl of plasma into a 100µl sample loop in the loading position. To begin the run and load 100 µl onto the column switch the loop to the inject position.
6. Run the FPLC program at a constant flow rate of 0.5 ml/min using the volume control. Set the fraction collector for 0.5 ml/tube. Discard the first 20 fractions (10ml). Start collecting from fraction numbers 21 to 68 (i.e. 48 fractions are collected). After the final fraction is collected, flush the column with an additional 26 ml (the total volume for the run is 60ml and the run time 2h).
7. When the program is finished, the next sample can be loaded without further washing. However, if triglycerides in the column fractions are to be measured an intermediate wash step must be performed to get rid of free glycerol (see step 3 above) before loading the next sample.

Reagent Preparation: *(This area may have several different preparations with the table of contents below.)*

[FPLC Buffer](#)

FPLC Buffer:

Reagents and Materials

Reagent/Material	Quantity Required	Vendor	Stock Number

Procedure