SOP 3.6 Protein Extraction from Blood

SOP Number: 3.6
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for protein extraction from whole blood.

Responsibility

It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

General precautions

- Prior to commencing read and understand the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (chloroform, 2-mercaptoethanol, CTAB, isopropanol and isoamyl alcohol).
- Protective clothing, including laboratory coat, gloves and protective glasses, must be worn at all times when performing this procedure.
- Always use chloroform and 2-mercaptoethanol in a fume hood. When working in the fume hood, ensure the fan is on and the sash is lowered to the correct level as indicated by the arrows on the frame and sash.

Considerations

- Protein solutions should be prepared in high concentration, preferably 1mg/mL or greater. The high concentration tends to stabilise the protein's native structure as well as inhibiting protein "sticking" to otherwise inert surfaces such as glass and plastic. If high concentrations of the native protein are unrealistic, addition of a second inert protein at high concentration will help prevent losses of protein on inert surfaces. Rinsing with EDTA solution prior to deionised water removes any possibility of contamination by metal ions.

- Vigorous shaking or stirring (e.g. vortex) can generate shear forces that in certain instances can destroy biological activity.
- When storing proteins antibacterial agents such as sodium azide can be added to inhibit bacterial growth. The addition of stabilisers such as glycerol helps prevent damage to the protein during freezing and thawing. Typical concentrations for glycerol are 10% to 50%. Although stable while frozen, repeated thawing and freezing of a sample can lead to degradation and loss of activity.

Equipment/reagent requirements

- Hanks balanced salt solution
- Ficoll density gradient solution
- Cell lysis solution
- Protease inhibitor solution
- A refrigerated centrifuge capable of 12,000g
- A vortex mixer
- -80^{o}C Freezer
- A spectrophotometer capable of reading 260 and 280nm /Nanodrop

Procedure

1. Cells are isolated as follows;
   a) Dilute 10mL of blood / cell pellet with 10mL of 1X Hanks balanced salt solution (HBSS)
   b) Layer this solution over 10mL of a ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) and centrifuge in a 50mL disposable plastic centrifuge tube for 15 min at RT at 2,000g with BRAKE OFF.
   c) A white band containing peripheral lymphocytes should be visible in each tube.
   d) Remove and discard the sample above this and transfer the white band to a fresh 15mL centrifuge tube.
   e) Wash the cells by adding 10mL of HBSS, mix thoroughly, and recover the cells by centrifugation for 10min at RT at 2,000g

2. Discard the supernatant and resuspend the cell pellet in Cell Lysis Buffer. The volume of CelLytic^{TM}-M lysis/extraction reagent (Sigma Aldrich) to be added to the cells varies according to cell size and protein concentration required. In general: 125µl CelLytic^{TM}-M is recommended for 10^{6}-10^{7} cells.

3. A commercial protease inhibitor solution (for example P3840 Sigma-Aldrich) may be added to the CelLytic™-M reagent to reach a final concentration of 1X in the buffer.

4. Centrifuge the lysed cells for 15 min at 12,000-20,000g at 4°C to pellet the cellular debris.

5. Remove the protein-containing supernatant to a 0.5mL cryostorage tube chilled to 4°C and store at -80°C.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.