Dublin Academic Medical Centre

Standard Operating Procedure
Dublin Academic Medical Centre
UCD Clinical Research Centre

SOP Number	**5.4**
Version Number	**1**
SOP Title	**Extracting Protein from Blood**

	NAME	TITLE	DATE
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Purpose

To outline the laboratory procedure for extracting total protein from blood.

Specific procedure

1. Know the location of the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (chloroform, 2-mercaptoethanol, CTAB, isopropanol and isoamyl alcohol). Read and become familiar with the safe use of these chemicals. If you have a question or concern regarding health or safety with respect to a specific chemical, consult the laboratory supervisor, or the principal investigator before proceeding.

2. Always use protective clothing when performing this procedure. This means wear a laboratory coat, goggles.

3. Always use chloroform and 2-mercaptoethanol in a fume hood. When working in the fume hood, always be certain the fan is on and the sash is lowered to the correct level as indicated by the arrows on the frame and sash.

4. Whenever you have a question or concern regarding health or safety with respect to a specific procedure, consult the laboratory supervisor or the principal investigator before proceeding.

5. For protein extraction, the molecular biology laboratory use a specific Lysis Buffer provided by the Sigma-Aldrich company, the CellLytic buffer. This buffer is useful for the protein extraction from adherent and non-adherent cell. Different projects can involve particular and singular protein extraction kit which works in the same way.

    a) The volume of CelLyticTM-M lysis/extraction reagent to be added to the cells varies according to cell size and protein concentration required. In general: 125 µl CelLyticTM-M is recommended for 10e6-10e7 cells, collected by centrifugation.
    b) Protease Inhibitor cocktail may be added to the CelLyticTM-M reagent
    c) Wash cells and treat with cell lysis buffer.
    d) Incubate the cells for 15 minutes on a shaker.
    e) Collect cell lysate.
    f) For adherent cells: remove cells from plates (cell scraping might increase total protein yield).
    g) Centrifuge the lysed cells for 15 minutes at 12,000- 20,000 X g to pellet the cellular debris.
    h) Remove the protein-containing supernatant to a chilled test tube.

    Note: Lysate preservation requires low temperatures. Therefore, for long term storage it is recommended to store lysate at –70 °C.

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Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.