Dublin Academic Medical Centre

Standard Operating Procedure
Dublin Academic Medical Centre
UCD Clinical Research Centre

SOP Number	**5.1**
Version Number	**1**
SOP Title	**DNA Extraction from Blood**

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Purpose

To outline the laboratory procedure for extracting DNA 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.

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DNA extraction from blood using a standard chemical method:

Blood should be collected into heparinized vacutainers. Ideally, it should be processed immediately but can be stored overnight at 4oC

1. Dilute 10ml of blood with 10ml of HBS

2. Layer this over 5 ml Histopaque and centrifuge in a 15ml disposable plastic centrifuge tube for 15 min at room temperature at 2000g

3. A white band containing peripheral lymphocytes should be visible in each tube.

4. Remove and discard the sample above this and transfer the white band to a fresh 15ml centrifuge tube.

5. Wash the cells by adding 10ml of HBS, mix thoroughly, and recover the cells by centrifugation for 10min at room temperature at 2000g

6. Discard the supernatant and resuspend the cell pellet in 20ml extraction buffer

7. Add proteinase K to 1003g/ml(1003l of stock) and gently swirl the beaker to mix the components. Incubate the beaker at 37oC for at least 3h, preferably overnight, with gentle agitation. This can be achieved using a

shaking water bath or by occasional swirling by hand. The solution should be reasonably clear and visous at the end of the incubation. More Proteinase K may be added to achieve this.

8. Add 10ml of equilibrated phenol and seal the beaker with parafilm. Gently swirl by hand for 10-15min to mix the two phases. The larger the surface is available, then easier this will be. Ideally you should generate an emulsion at this stage. It may be necessary to transfer the mixture to a large container to achieve this.

9. Transfer the mixture to a 50ml disposable plastic tube and centrifuge at 1500g for 10min at room temperature to separate the two phases.

10. Remove the lower phenol phase by gentle aspiration through a Pasteur pipette attached to a vacuum line through a side arm flask. The pipette should be lowered into the lower phenol phase with the vacuum line clamped until the thread of viscous DNA has detached from the pipette tip. Slowly unclamp the vacuum line and allow the phenol phase to run into the flask. Once all of the phenol has been removed, the vacuum line is again clamped and the pipette is removed. Alternatively, the aqueous phase can be removed with a wide-bore pipette. However, care must be taken not to disturb the interface and when the DNA is very viscous this is hard to achieve.

11. At this stage two routes to recovery of DNA are available 1: Dialyze the aqueous phase against 1000vol of TE. This should be performed for 30min at room temperature to prevent SDS precipitation in the sample followed by overnight at 4oC. Allow room for expansion in the dialysis bag. 2: Transfer the aqueous phase to a fresh beaker and add sodium acetate to 0.3M. Mix by gentle swirling. Add 2 vol of absolute ethanol and mix by gentle swirling. The DNA will begin to precipitate almost immediately in a strand complex. Initially this will be glass-like but it will begin to attain a white appearance as he precipitation proceeds. Hook out the DNA strands using Pasteur pipette with a sealed U-shaped en before they attain too white in appearance. Dip the DNA in 70% ethanol for a few seconds and allow to air dry for a few minutes. Transfer the DNA to 1-3ml TE. Gently wet the DNA in the liquid and allow it to fall off the pipette tin onto the surface of the liquid. Do not shake violently to achieve this. Leave to dissolve overnight at 4oC. If this proves difficult, incubate the tube overnight at room temperature on a gently rocking table or rotating wheel.

12. The absorbance of the DNA at 260nm and 280nm should be measured using quartz cuvets. The 260/280 ratio should be >1.8. An A260 of 1.0 in a 1-cm light path is equivalent to a DNA concentration of 505g/ml. Store the DNA at -80oC.

13. An aliquot of the DNA should be analyzed by electrophoresis through a 0.3% agarose gel. The prepared DNA is normally at least 100kbp and preferably exceeds 200kbp.

    DNA extraction can also be performed using the QIAmp DNA blood mini kits provided by Qiagen. This method is faster and easier than the previous one. The procedure is the same (lyses, binds, wash1, wash2, elutes) for both the extractions; check and use the Qiagen handbook to follow the procedure for the DNA extraction from blood.

Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.