



## AutoGen® AUTOMATED DNA EXTRACTION FROM BLOOD or LYMPHOCYTES with PHENOL/CHLOROFORM

The cell and nuclear membranes are destroyed by the combined action of SDS and proteinase K. The cell debris from lysis, mainly protein in nature, is captured by organic solvents such as phenol and chloroform. The DNA, which is insoluble in the organic phase, is present in the non-miscible aqueous phase. The DNA is precipitated, washed, dried and redissolved in solution in an aqueous buffer.

### Equipment and Materials

- 50 ml SARSTEDT tubes
- Nunc tubes 4.5 ml
- An aspiration system including : a pump, a vacuum trap, a bottle.
- 2-3 DONASET tubules and 1-2 needles (lumbar puncture type)
- Centrifuge
- Agitator incubator at 37°C
- AutoGen® extractor, NA-2000 (Geneworx)
- Hole tubes units, AGPT 5000 (Geneworx)
- Nunc tubes 4.5 ml
- Disposable gloves

### 1. REAGENTS, MEDIA & SOLUTIONS

To be kept at room temperature :

TRIS Base PM 121.1g (1kg)  
Magnesium Chloride PM 203.3 g (1kg)  
NaCl PM 58.44 g (1kg)  
EDTA PM 372.4 g (1kg)  
HCl 37% (1L)  
NaOH (PASTILLES) (500 g)  
SDS 20% (500 ml)  
Absolute ethanol  
Phenol / KOAc  
Ethanol / Butanol  
Propanol  
TE 10<sup>-1</sup>  
Ethanol 70%

AUTOGEN  
AUTOGEN

To be stored at 4°C :

Proteinase K (500 mg)

### 2. PREPARATION OF STOCK SOLUTIONS

These stock solutions should be stored at +4°C and are used for the preparation of working solutions.

#### TRIS 2M pH 7.6

- Weigh 242.2g of TRIS Base
- 800ml distilled water
- Adjust to pH 7.6 with HCl ( $\cong$  95 ml)
- Distilled water to 1000ml



#### **MgCl<sub>2</sub> 1M**

- Weigh 203.3g of MgCl<sub>2</sub>
- Distilled water to 1000 ml

#### **NaCl 3M**

- Weigh 175.32g of NaCl
- Distilled water to 1000 ml

#### **EDTA 0.4 M PH 8**

- Weigh 148.96 g of EDTA
- Weigh 18 g of NaOH
- Water 800 ml
- After dissolving, adjust to pH 8 by adding NaOH, pastille by pastille
- Distilled water to 1000 ml

### **3. PREPARATION OF WORKING SOLUTIONS**

These working solutions are prepared in sterile graduate cylinders using sterile distilled water (cover with parafilm to mix) and stored at +4°C.

#### **SLR**

- |                        |         |
|------------------------|---------|
| - TRIS 2M pH 7.6       | 10 ml   |
| - MgCl <sub>2</sub> 1M | 10 ml   |
| - NaCl 3M              | 6.6 ml  |
| - Distilled water to   | 2000 ml |

#### **SLB**

- |                                      |         |
|--------------------------------------|---------|
| - TRIS 2M pH 7.6                     | 10 ml   |
| - EDTA 0.4 M pH 8                    | 50 ml   |
| - NaCl 3M                            | 34 ml   |
| - Add 500 ml water, shake (parafilm) |         |
| - SDS 20%                            | 20 ml   |
| - Distilled water to                 | 2000 ml |

#### **SLB Solution / Proteinase K**

##### **Δ Gloves must be worn**

Dissolve 500 mg of proteinase K powder (stored at +4°C) in 10 ml SLB. Pour this solution in a 2 L graduated cylinder containing 500 ml of SLB, rinse the flask with 10 ml of this mixture, and adjust the volume of SLB to 1250 ml.

Freeze this solution at -20°C in small aliquots.

#### **TE 10-1**

- |                      |         |
|----------------------|---------|
| - TRIS 2M pH7.6      | 5ml     |
| - EDTA 0.4 M pH8     | 2.5 ml  |
| - Distilled water to | 1000 ml |

#### **Saline**

- |                      |         |
|----------------------|---------|
| - NaCl 3M            | 50 ml   |
| - Distilled water to | 1000 ml |

#### **Ethanol Solution 70 %**

- |                       |         |
|-----------------------|---------|
| - TE 10 <sup>-1</sup> | 600 ml  |
| - Absolute ethanol to | 2000 ml |



## Procedure

### 1. LYSIS OF RED BLOOD CELLS

#### 1.1 Discarding plasma

- Pool the tubes of blood in a 50 ml GREINER tube
- Centrifuge for 10 min at 2000 rpm
- Aspirate the plasma without touching the leukocyte layer (buffy coat)
- Mix the red blood cells and leukocytes

#### 1.2 Lysis of red blood cells

- Lyse in a 50 ml final volume with a solution of SLR
- Centrifuge for 10 min at 2000 rpm
- Aspirate about 45 ml of lysed red blood cells. Re-suspend the pellet using a sterile pipette, and make up to 50 ml with SLR
- Centrifuge for 5 min at 2000 rpm
- Remove the supernatant
- If red blood cells still remain, resuspend the cellular pellet in 5 ml of SLR
- Proceed to a third centrifugation and remove the supernatant
- Freeze the pellet at -80°C

### 2- LYSIS OF LEUKOCYTES

- Defrost the pellet in a water bath at 50°C for one minute
- Lyse the leukocytes in one volume of SLB + proteinase K (see 3.3); the volume to use depends on the size of the pellet:
  - very small pellet: 5 ml
  - small pellet: 10 ml
  - nice pellet: 15 ml
- Homogenize the tube with gentle rotation
- Incubate overnight at 42°C with agitation

### 3- EXTRACTION OF PROTEINS by AutoGen®

- Check the level of reagents in the apparatus
- Place the lysate in the extractor tubes
- Turn on the apparatus; the cycle lasts for about one hour, during which the lysate is mixed with the phenol/chloroform/potassium acetate reagent and the DNA is then precipitated with the ethanol/butanol mixture. The DNA is then collected in new extractor tubes
- Transfer the precipitated DNA "medusa" to a 4.5 ml Nunc tube
- Wash the "medusa" with 1 ml of isopropanol, then 3 times with 1 ml of 70% ethanol
- Let the medusa dry until it becomes translucent
- Add TE 10<sup>-1</sup>(0.1 to 1 ml depending on the size of the medusa)
- Release the medusa by shaking the tube
- Incubate overnight at 37°C with rotation
- Leave the tubes at 4°C for about one week
- Then you can store the tube at -20°C for a long period of time

### 4 - CHECK QUANTITY/QUALITY OF DNA (see QUALITY CONTROL OF DNA protocol)