

DNA Analyst Training Laboratory Training Manual

Protocol 3.01 DNA Isolation: General Information



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S
DNA
INITIATIVE



INTRODUCTION

DNA profiles obtained from biological evidence provide information as to the source of the sample. The goal of DNA isolation is to recover/extract high molecular weight DNA in sufficient quantities. Through the use of standard procedures, DNA is isolated from nucleated cells from various biological specimens. Cell lysis is achieved by the addition of proteinase K, DTT, SDS, and EDTA. Phenol and chloroform removes the DNA from the cellular materials. This DNA is then further purified through microcon filtration.

SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents. Wear appropriate personal protective equipment and use the fume hood when using these chemicals.

Chloroform is toxic and a suspected human carcinogen. It is harmful if inhaled, ingested or exposed to the eyes or skin.

Hydrochloric Acid is a corrosive chemical that causes severe burns if inhaled, ingested or exposed to the eyes or skin and may cause permanent tissue damage.

Phenol is a toxic and corrosive chemical and a suspected carcinogen. It is harmful or fatal if ingested, inhaled, or exposed to the eyes. Special protection: use of a local exhaust hood is required; use chemical resistant protective gloves (LATEX gloves are NOT a sufficient barrier of protection) such as neoprene or rubber.

Sodium Dodecyl Sulfate (SDS) is harmful if inhaled or ingested. It potentially causes skin and eye burns. Special protection: Wear a dust mask or respirator; use of a fume hood is required.

Sodium Hydroxide is a corrosive chemical which may be fatal if swallowed or absorbed through the skin. It causes severe eye, skin, digestive and respiratory tract burns.

8-Quinolinol is irritating to the eyes, skin, mucous membranes and upper respiratory tract. Laboratory experiments have shown mutagenic effects.

Xylene is harmful if ingested, inhaled, or exposed to the eyes or skin and has caused adverse reproductive and fetal affects in animals.

Xylene substitute may be harmful if high vapor concentrations are inhaled.

PREPARATIONS

DNA Isolation Reagents: Equivalent preparations may be purchased commercially, if available.

Chloroform/Isoamyl Alcohol (24:1)

Chloroform 96 ml

Isoamyl alcohol 4 ml

Prepare in fume hood.

390 mM DTT

Dithiothreitol 620 mg

ddi H₂O (or equivalent) 10 ml

Aliquot into convenient size volumes and freeze. Remix after thawing.

500 mM EDTA pH 8.0

EDTA-2H₂O·Na₂ 930.5 g

ddi H₂O (or equivalent) 4.0 l

NaOH pellets 75-100 g

When fully dissolved, add additional NaOH to bring the pH to 8.0

(EDTA does not go into solution

until the pH nears 8.0.) Adjust the volume to 5.0 liter. Autoclave.

Phenol/Chloroform/Isoamyl Alcohol

Buffer-Saturated Phenol 100 ml

Chloroform 96 ml

Isoamyl alcohol 4 ml

8-Quinolinol (optional) 200 mg

Prepare in fume hood. Store in glass bottles.

Proteinase K (20µg/µl)

Proteinase K 500 mg

ddi H₂O (or equivalent) 25 ml

Aliquot immediately into convenient size volumes and freeze.

Remix after thawing.

20% SDS

SDS 1000 g

ddi H₂O to 5 liters

Heat gently to about 65°C and stir to dissolve. Use a nuisance mask.

Prepare reagent in the fume hood.

Stain Extraction Buffer (SEB)

1.0 M Tris, pH 8.0 10 ml
500 mM EDTA, pH 8.0 20 ml
NaCl 5.84 g
20% SDS 100 ml

Add ddi H₂O (or equivalent) to 1.0 liter.

TE⁻⁴

1.0 M Tris pH 8.0 10 ml
500 mM EDTA, pH 8.0 0.2 ml

Add ddi H₂O (or equivalent) to 1.0 liter. Autoclave.

TNE

1.0 M Tris, pH 8.0 1.0 ml
NaCl 584 mg
500 mM EDTA, pH 8.0 400 :l

Add ddi H₂O (or equivalent) to 100 ml.

1.0 M Tris, pH 8.0

Tris base 121.1 g
ddi H₂O (or equivalent) 800 ml

Adjust to pH 8.0 with approximately 45 ml concentrated HCl. Add ddi H₂O to 1.0 liter. Autoclave.

INSTRUMENTATION

Standard Laboratory Instrumentation

MINIMUM STANDARDS & CONTROLS

A manipulation blank consisting of a sterile swab, must be processed with each set of unknowns for every extraction protocol followed. One manipulation blank can be used for multiple cases. Process the manipulation blank last with each set of samples. The purpose of this control is to ensure that contamination has not occurred due to the manipulation of the sample or the reagents used in the procedure. Whenever an additional manipulation is done on a sample (i.e., ten second injections or re-concentration of the DNA) the same manipulation must be done to the manipulation blank.

PROCEDURE OR ANALYSIS

Refer to the appropriate procedure for the type of sample from which DNA is being extracted.

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