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PROCEDURE FOR DMPC TREATMENT OF GLASSWARE AND PLASTICWARE

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1. PRINCIPLE:

Techniques and experiments in molecular biology employed in the study of infectious diseases greatly rely on information obtained by studying the genetic material from pathogens or of infected individuals. It is important that the laboratory work areas are clean and free from nucleases that can degrade DNA or RNA.

Aside from work areas, commonly used materials and supplies must also be free from DNases and RNases. These nucleases are present in most biological excretions, including sweat and saliva. Without proper management, normal human handling of materials and supplies can contaminate these with nucleases and may affect downstream processing of nucleic acids. It is important to note that RNA is more sensitive to destruction and degradation because of its molecular structure, and as such, more should be given to handling RNA-extracted samples.

Commonly used glassware are routinely decontaminated and washed before using again. Plastic supplies purchased from manufacturers are generally sterile, single-use only and disposable. For non-disposable plasticware, decontamination and sterilization is done differently since certain plastics cannot be sterilized by autoclaving. Both glassware and plastic supplies must be DMPC-treated to deactivate nucleases before using in any laboratory procedure, except if the purchased plastic supplies are already DNase-free and RNase-free as indicated from the supplier or manufacturer. DMPC inactivates enzymes by reacting with the amine, hydroxyl and thiol groups of proteins by methoxy carbonylation of the histidine residues. Treatment of glassware and plastic supplies with DMPC effectively renders your nucleases nonfunctional, and safe for use with nucleic acid processing.

DMPC is a safer alternative to the more popularly used DEPC, which is a known carcinogen.

2. SUPPLIES/MATERIALS/EQUIPMENT:

a. SUPPLIES

i. Glassware
ii. Non-disposable plasticwares

b. MATERIALS

i. 0.1 M NaOH
ii. 1 mM EDTA
iii. 0.1% DMPC
iv. 100% ethanol
v. Chloroform

c. EQUIPMENT

i. Autoclave

ii. Drying oven
iii. Hot plate with magnetic stirrer

3. PROCEDURE:

1.1. PRE-PROCEDURE

1.1.1. **SAFETY PRECAUTIONS:**

1.1.1.1. Wipe surfaces of benches and work stations with 100% ethanol to kill and remove contaminating microbes and their exogenous RNases

1.1.2. **HANDLING:**

1.1.2.1. Wear gloves in handling DMPC, NaOH, EDTA and chloroform

1.1.2.1.1. Please refer to LRD-MBL-WI-101: Donning and Doffing of Gloves

1.2. PROCEDURE PROPER

1.2.1. **PREPARATION OF STOCK AND WORKING REAGENTS**

1.2.1.1. Preparation of DMPC-treated water

1.2.1.1.1. Add 0.1mL DMPC to 100mL water

1.2.1.1.2. Incubate for 12 hours at 37°C

1.2.1.1.3. Autoclave for 15 minutes at 121°C at 15psi

1.2.1.2. Preparation of 1M NaOH stock solution

1.2.1.2.1. Dissolve 40g of solid NaOH in a liter of DMPC-treated water or RNase-free water

1.2.1.3. Preparation of 0.1M NaOH working solution

1.2.1.3.1. Dilute 1-part 1M NaOH stock solution in 9 parts DMPC-treated water or RNase-free water

1.2.1.4. Preparation of 1M EDTA stock solution

1.2.1.4.1. Weigh 292.24g EDTA

1.2.1.4.2. Transfer solid EDTA onto a beaker

1.2.1.4.3. Add 800mL of DMPC-treated water or RNase-free water

1.2.1.4.4. Place a magnetic stirrer in the solution

1.2.1.4.5. Transfer beaker on a magnetic hot plate and stir the solution

1.2.1.4.6. Add 0.1M NaOH to the solution drop-wise while stirring
NOTE: EDTA will only dissolve at pH 8.0

1.2.1.4.7. Once EDTA dissolves, add DMPC-treated water or RNase-free water until the volume is 1L.

1.2.1.5. Preparation of 1mM EDTA working solution

1.2.1.5.1. Dilute 1-part 1M EDTA stock solution in 999 parts DMPC-treated water or RNase-free water

1.2.2. **TREATMENT OF GLASSWARE AND AUTOCLAVABLE PLASTICWARE**

1.2.2.1. Clean glassware and autoclavable plastic ware with a detergent

1.2.2.2. Rinse thoroughly

1.2.2.3. Fill glassware and autoclavable plasticware with 0.1% DMPC

1.2.2.4. Allow to stand overnight (12 hours) at 37°C
1.2.2.5. Sterilize glassware and plasticware in an autoclave for 15 minutes at 121°C at 15psi
1.2.2.6. Oven-dry glassware and plasticware at 70°C

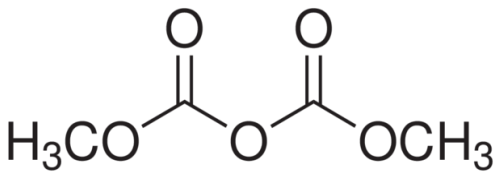
1.2.3. **TREATMENT OF NON-DISPOSABLE NON-AUTOCLAVABLE PLASTICWARE**
1.2.3.1. Thoroughly rinse plasticware with 0.1M NaOH
1.2.3.2. Thoroughly rinse plasticware with 1mM EDTA
1.2.3.3. Thoroughly rinse plasticware with DMPC-treated water or RNase-free water
1.2.3.4. Alternatively, if the plasticware is chloroform-resistant, thoroughly rinse plasticware with chloroform to deactivate RNases
1.2.3.5. Oven-dry plasticware at 70°C

1.3. POST-PROCEDURE

1.3.1. **STORAGE:**
1.3.1.1. Store DMPC-treated glassware and plasticware in designated RNase-free materials and supplies storage areas

1.3.2. **WASTE DISPOSAL:**
1.3.2.1. EDTA, NaOH, ethanol waste may be disposed in the sink
1.3.2.2. DMPC waste must first be deactivated by autoclaving before disposal in the sink
1.3.2.3. Chloroform waste must be disposed as an organic solvent and sent to an approved chemical treatment facility

Appendix 1 DMPC (dimethyl pyrocarbonate)



CAS Number:	4525-33-1
ESIS EC# (EINECS):	224-859-8
Molecular Weight	134.08782000
Formula:	$C_4H_6O_5$
Synonyms:	methoxycarbonyl methyl carbonate, dimethyl dicarbonate (DMDC), dimethyl oxydiformate, dicarbonic acid dimethyl ester, velcorin
Soluble in:	water, toluene
Density:	1.25g/mL at 25°C
Boiling point:	45-46°C/5 mmHg
Application:	Methylates histidine residues of enzymes, inhibiting enzymatic activity