ACGT MICROARRAY FACILITY STANDARD OPERATING PROCEDURE		
TITLE: Cassava RNA isolation		**PAGE: 1 of 2**
SOP#: MA003	**REVISION LEVEL: .1**	**DATE: 2 November 2005**
AUTHOR: E. Pierce	**REVIEWERS: Sanushka Naidoo**	

1. PURPOSE

This procedure describes RNA isolation from cassava leaf tissues using a high molecular weight polyethylene glycol method.

(Modified from Plant Molecular Biology Reporter (2000) 18:369-376)

2. SCOPE

This RNA isolation procedure may be applied for plant species rich in polysaccharides and polyphenols.

3. MATERIALS

GHCL buffer: 6.5M guanidium hydrochloride, 100mM Tris-HCl (pH8.0),

0.1M Sodium acetate (pH5.5), 0.1M β-mercaptoethanol

HMW-PEG (high-molecular weight polyethylene glycol) (20 000 mol wt,Sigma)

1M Sodium Citrate (pH4.0)

2M NaCl

PCI (phenol,25/chloroform,24/isoamyl alcohol,1)

Isopropanol

75% v/v Absolute ethanol

DEPC (Sigma, catalogue number D5758)

50ml sterile plastic screw-cap centrifuge tubes (Falcon, Becton Dickinson, catalogue number 352070)

Liquid Nitrogen

Rnase Away® (Molecular BioProducts, catalogue number #7003)

Pestle and mortar

4. PREPARATION

When working with RNA, ensure all bench surfaces are wiped with RNAse Away and gloves are used at all times. RNAses are very stable and active enzymes that generally do not require cofactors to function. Care should be taken not to introduce RNAses into the RNA extraction procedure.

Prepare a 0.1% (v/v) solution of DEPC water as follows:

In the fume hood, add 1ml of DEPC to 1L of ddH_2O in a schott bottle.

Allow the mixture to stir overnight.

Autoclave the DEPC water for 20min.

Prepare 75% EtOH in DEPC water.

5. PROCEDURE

Grind 1g of leaf tissue in liquid nitrogen with a pestle and mortar.
Add ground tissue to 5ml GHCL buffer.

Add 0.1g HMW-PEG.

Centrifuge at 8500 rpm for 8 min at RT.

Transfer supernatant to new tube.

Add: 0.1ml (1M) Sodium citrate (pH4.0)
0.2ml (2M) NaCl
5ml PCI (25/24/1)

Mix and incubate for 10 min at RT.

Centrifuge at 9000 rpm for 10 min.

Transfer supernatant to new tube.

Add 5ml Isopropanol.

Mix and incubate for 1h at -20°C.

Centrifuge at 10 000 rpm for 30 min.

Wash pellet with 75% v/v ethanol.

Centrifuge at 10 000 rpm for 10 min.

Air-dry pellet for 5-10 min.

Resuspend in 100µl RNase- free (DEPC) water.