

## Solutions

### **Extraction buffer (100 mM Tris-HCl, 50 mM EDTA, 50 mM NaCl, 10% SDS - pH 7.5, ca. 32 mL H<sub>2</sub>O)**

For 100 samples (50 mL solution)

10 grams SDS (Sodium Dodecyl Sulfate,  $d = 1.01 \text{ g/cm}^3$ )

146.1 mg Sodium Chloride (NaCl,  $mw = 58.4 \text{ g/mol}$ )

930.6 mg EDTA (Ethylenediaminetetraacetic Acid Disodium Salt dihydrate,  $mw = 372.24 \text{ g/mol}$ )

Add 20 mL deionized H<sub>2</sub>O

5 mL Tris-HCl pH 8.0 (1 molar- kept in fridge)

Fill to 50 mL with deionized H<sub>2</sub>O

Auto-clave on 'Liquid' setting for 15 minutes.

Dissolution may require heat and stirring (3 & 4 settings respectively, ca. 15 min.)

### **10% CTAB solution (20 mM Tris-Cl pH. 8.0, 1.4 M NaCl, 10 mM EDTA pH 7.5, 10% CTAB, 5% PVP, 40 mL DiH<sub>2</sub>O)**

For 100 samples (50 mL solution)

add ~30 mL deionized H<sub>2</sub>O,

1 mL Tris-HCl pH 8.0 (1 molar- kept in fridge; 2-Amino-2-(hydroxymethyl)propane-1,3-diol)

4.08 g Sodium Chloride (NaCl,  $mw = 58.4 \text{ g/mol}$ )

4 mL EDTA pH 7.5 (0.125 molar – kept in fridge; 2,2',2'',2'''-(Ethane-1,2-diyl)dinitrilo)tetraacetic acid)

5 g CTAB (hexadecyl(trimethyl)ammonium bromide,  $mw = 364.45$ , FYI this is 274 mM)

Auto-clave on 'Liquid' setting for 15 minutes.

2.5 g PVP-40 (1-ethenylpyrrolidin-2-one) – add after autoclave

Fill to 50 mL with deionized H<sub>2</sub>O

Dissolution of PVP will require 2-3 hrs, at 65°C with stirring. Before use allow one hour of stirring and heat to resuspend all salts in the solution.

### **Sodium acetate solution (3mM)**

For 100 samples (10 mL solution)

20.4 mg Sodium Acetate trihydrate ( $mw = 136.08 \text{ g/mol}$ )

to 50 mL deionized H<sub>2</sub>O

Auto-clave on 'Liquid' setting for 15 minutes.

### **Phenol-chloroform Isoamyl alcohol (25:24:1) Saturated with 10 mM Tris pH 8.0, EDTA**

For 100 samples (50 mL solution) (no need to make, is bought)

25 mL Phenol

24 mL Chloroform (Trichloromethane)

1 mL Isoamyl alcohol

### **Literature cited**

Lalmangaibi, R., Ghatak, S., Laba, R., Gurusubramian, G., Jumar, N.S. *Protocol for Optimal Quality and Quantity Pollen DNA Isolation from Honey Samples*. 2014. Journal of Biomolecular Techniques 25:92-95

Guertler, P., Eicheldinger, A., Muschler, P., Goerlich, O., Bursch, U. *Automated DNA extraction from pollen in honey* 2014. Food Chemistry 149:302-306