# DNeasy Blood & Tissue Kit.

**DNA extraction Protocol (adjusted to *Timema bartmani*)**

##  TIPS

* Use always an aliquot of the reagent, never pipet directly from the stock.
* Label the aliquots in the container and in the lid with reagent name and date.

## BEFORE STARTING the First part

### Place **ATL** aliquot container in the incubator

### **Preheat incubator** at **56º C** for ≤3 hour incubation; 37 or 56 ºC if overnight.

## FIRST PART

### Take 24 samples from the freezer: 2 mL Eppendorf centrifuge tubes with dry *Timema sp.* tissue, not in ethanol.

### Burn forceps with the cook lighter to sterilize

### Place one clean **metal bead** in the lid of each tube and close it.

### Place 12 samples (distribute the weight) in each plate of the **Tissue Lisser** (dark room). Secure them and run it at **30.0 ps x 1 min.**

 **Centrifuge** tubes to clean lid (6000 rps x 15S usually is more than enough).

### Add **180 µL** of **ATL** to each tube

### Add **20 µL** of Proteinase **K** to each tube

 Vortex (mix well)

 Place the tubes well distributed in a rack

 Wrap it with paper

 Write name, group, date, temperature of incubation and time interval of incubation.

 Wrap it with film

 Secure it to the roaster of the Incubator

 Activate rotation

### **Incubate at 56ºC for at least 3 hours** or at 37ºC if is going to be for long (overnight).

**Use the extra time of incubation to number 24 spin columns**

**and label 24 1.5 mL Eppendorf centrifuge tubes.**

## BEFORE STARTING the Second part

### Place **AL** aliquot container in the incubator

## SECOND PART

**Switch-off** the **Incubator**

**Centrifuge** tubes to clean lid (7000 rps x 15S usually is more than enough).

Take **AL** from the **Incubator**

### Add **200 µL** of **AL** to each tube

 Shake gently (If lid is not clean spin gently)

### Add **200 µL** of **Ethanol 100%** to each tube

 Shake gently (If lid is not clean spin gently)

### **Pipet all** liquid **to** the sorted and numbered **mini spin columns** (~500 – 600 µL)

### Centrifuge at **8000 rpm x 1.15 min** (Prepare new collection tubes)

 Place spin-columns in new collection tubes

### Add **500 µL** of **AW1** to each tube

### Centrifuge at **8000 rpm x 1.15 min** (Prepare new collection tubes)

 Place spin-columns in new collection tubes

### Add **500 µL** of **AW2** to each tube

### Centrifuge at **14000 rpm x 3.15 min** (Prepare labelled 1.5 Eppendorf tubes)

 Place spin columns in 1.5 mL Eppendorf centrifuge tubes (LABELLED AND SORTED!)

### Add **65 µL** of **AE** to each tube

### Incubate at room temperature for **30 min**

### Centrifuge at 8000 rpm x 1.15 min

### Add **45 µL** of **AE** to each tube

### Incubate at room temperature for **30 min**

### Centrifuge at 8000 rpm x 1.15 min

Discard mini spin columns. Close the Eppendorf tubes and keep them in the freezer if you are not going to use the DNA in the following days.

**Clean your mess: You can wash the dirty Metal Beads with 10% Bleach**