



## Isolation of Hair, Nail and Feathers using Tissue DNA Kit

### Protocol For Isolation of DNA From Hair, Nails and Feathers:

1. Cut the sample into small pieces (0.5-1 cm) and transfer it to a 1.5 mL centrifuge tube.  
**Tip:** For hair, cut from base of hair; for feathers: select the primary feathers. (Large birds, secondary tail or breast feather can be use).
2. Add 250  $\mu$ L TL Buffer, 25  $\mu$ L OB protease and 20  $\mu$ L 1M DTT. Mix thoroughly by vortexing. Incubate 30 min at 60°C with occasional mixing.
3. Add 250  $\mu$ L Buffer BL to the sample, mix thoroughly by vortexing. Add 250  $\mu$ L absolute ethanol to the sample, mix thoroughly again by vortexing
4. Prepare the column by adding 100  $\mu$ l of Equilibration Buffer inserted in a 2 mL collection tube. Centrifuge at  $\geq 13,000 \times g$  for 20 seconds. Discard flow-through
5. Transfer the entire sample from step 3 into the column including any precipitate that may have formed. Centrifuge at 10,000x g for 30 to 60 seconds to bind DNA. Discard the flow-through
6. Add 500 $\mu$ l of Buffer HB and centrifuge at 10,000x g for 30 to 60 seconds. Discard the flow-through.
7. Add 700 $\mu$ l of DNA Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000x g for 30 to 60 seconds and discard the flow-through.  
**IMPORTANT:** DNA Wash Buffer must be diluted with absolute ethanol before use. See label for directions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.
- 8.. Repeat step 7 with an additional 700 $\mu$ l of DNA Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000x g for 30 to 60 seconds and discard the flow-through.
9. Centrifuge at maximum speed ( $\geq 13,000 \times g$ ) for 2 min to dry the HiBind® DNA Mini Column. This step is crucial for ensuring optimal elution in the following step.
10. Place the HiBind® DNA Mini Column into a sterile 1.5 ml microfuge tube, and add 100-200 $\mu$ l of preheated (70°C) Elution Buffer (10mM Tris,pH8.5). Allow to sit at room temperature for 3 minutes.
11. To elute DNA from the HiBind® DNA Mini Column, centrifuge at  $\geq 13,000 \times g$  for 1 min. Repeat the elution with a second 100-200 $\mu$ l volume of Elution Buffer