DetectX®

Hair, Feather and Nail Extraction Protocol

ARBOR ASSAYS®

For our DetectX™ Immunoassay Kits

For an accurate determination of concentrations of analytes in hair, feathers, nails and other hard solid samples, samples will need to be extracted prior to assaying. Human hair grows at about 1 cm per month and so a relatively small length (3 cm) will provide a profile of analyte concentration over the previous 3 months. This protocol deals with extraction from hair, but similar protocols can be used for other solid matrices.

For feather samples see: J. Avian Biol., 2011, 42, 247-252.
For nail samples see: Neuropsych. Dis. Treat., 2010, 6, 1-7.

Extraction of small molecular weight analytes such as steroids, prostaglandins, and peptides in an impervious matrix such as hair or nails must be undertaken with caution. Analytes become incorporated into hair through four general models: (1) active or passive diffusion from blood into growing cells in hair follicle, (2) diffusion from body secretion (sweat, sebum) during formation of hair shaft, (3) incorporation from deep skin compartments during hair shaft formation, and (4) external environmental sources after hair shaft formation. The following recommendations should allow these materials to yield acceptable analytical results.

This protocol is based upon the method of Davenport, MD., et al., Gen. and Comp. Endocrin., 2006, "Analysis of endogenous cortisol concentrations in the hair of rhesus macaques". 147, 255-261.

Materials Needed:

1. Sharp, fine surgical scissors
2. Isopropanol
3. Methanol
4. Retsch ball mill (mixer mill MM 200), http://www.retsch.com/, Germany, or similar
5. 10 mL stainless steel grinding jars
6. 12 mm stainless steel grinding balls
7. Speedvac™ or similar vacuum evaporator, or nitrogen for evaporation

PROCEDURE:

Sampling: Hair samples are obtained by shaving with clippers or hair is cut using fine surgical scissors from the posterior vertex region. The samples should be placed into small aluminium foil pouches for transport. Storage for most steroids can be at room temperature, other analytes may require storage at 4°C or lower.

Washing: Place 250 mg of hair into 15 mL conical polypropylene centrifuge tubes with screw caps. Add 5 mL of isopropanol and gently mix. Incubate for 3 minutes. Decant the isopropanol and repeat the wash twice more. Allow the washed hair to dry.

Extraction: The hair can be cut into sections representing different analysis time periods. Weighed samples are ground to a fine powder in the Retsch mill using one 12 mm steel ball at 30 Hz for 5 minutes. The steel ball is separated from the hair and 50 mg of ground powdered hair is placed in each centrifuge tube with 1 mL of methanol. Samples are rotated at room temperature overnight. The following day the tubes are centrifuged and the methanolic solution is drawn off and reserved. This can be saved in a tightly sealed vial at –20°C if necessary. Evaporate 0.5 mL of the methanolic solution either under nitrogen or using a Speedvac™ vacuum evaporator. The dried extracts are then reconstituted using the kit Assay Buffer in an appropriate volume to allow duplicate assay measurements. A small amount of organic solvent (such as methanol or ethanol) can be added prior to addition of kit Assay Buffer to help with solubility.