

## **Extraction Procedure for Cortisol, Corticosterone, Androstenedione, and Testosterone**

Includes: Cortisol, Corticosterone, Androstenedione, and Testosterone

Cat #'s: EA 65, EA 66, EA 68, and EA 78

### **Materials Needed:**

1. Ethyl Ether
2. Nitrogen Gas
3. 10x75 mm Glass Tubes

### **Procedure:**

1. Pipette 100  $\mu\text{L}$  of plasma into a glass tube (10x75 mm) and add 1 mL of ethyl ether.
2. Vortex the tube for 30 seconds, then allow the phases to separate.
3. Transfer the organic phase into a clean glass tube and evaporate the solvent with a stream of nitrogen gas.
4. Dissolve the residue in 100  $\mu\text{L}$  of diluted extraction buffer.
5. Dilute the extract 100 fold by adding 10  $\mu\text{L}$  of the above extract into 990  $\mu\text{L}$  of diluted extraction buffer.
6. Vortex and assay 50  $\mu\text{L}$  in duplicates.
7. Multiply the obtained values by 100 to give the final concentrations in ng/mL. If additional dilution is necessary, values must be multiplied by the additional dilution factor in order to calculate final ng/mL concentration. If the concentration is higher than the high range of the standard curve, the samples in #6 need to be further diluted and re-assayed.