UC Davis MMPC-Live Protocol
Corticosterone RIA

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(note that the following list should be linked to the appropriate location.)

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Summary:

The RIA (RadioImmuno Assay) is an assay method used for the quantification of various proteins. A standardized concentration of antibody specific to the analyte of interest is pipetted into test tubes. A standardized concentration of the analyte of interest that is labeled with a radioisotope (usually 125I) is added to the tubes. Standards and samples are pipetted into the tubes and the tubes are incubated. During the incubation period the standardized concentration of labeled analyte and the unknown concentration of analyte in the samples will compete for binding sites on the antibody. After the incubation period a precipitating reagent is added to the tubes and the bound antibodies are precipitated out using the double antibody-polyethyleneglycol precipitation technique. The tubes are centrifuged and the supernatant is aspirated and the precipitate is counted (using a gamma counter if 125I is used). If there is a low concentration of the analyte of interest in the samples, the labeled analyte will have a higher probability for binding to the antibody and thus there will be a higher count in the precipitate. If there is a high concentration of the analyte of interest in the samples there will be a lower count of labeled analyte in the precipitate. The counts and known concentrations of the standards are used to generate a standard curve, and the counts of the samples are used to interpolate quantitative concentrations for the analyte of interest from the standard curve.

Reagents and Materials: *(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)*

Reagent/Material	*Vendor*	*Stock Number*
Corticosterone Kit	MP Biomedicals	07120102

Protocol

1. Dilute samples 1:200 with steroid diluent.
2. Add 0.3 ml diluent to NSB tubes and 0.1 ml to zero tubes.

3. Add 0.1 ml of calibrators and diluted samples.
4. Add 0.1 ml of tracer to all tubes.
5. Add 0.1 ml of antibody to all tubes except Total Count and NSB tubes.
6. Incubate at RT for 2 hrs.
7. Add precipitating reagent and centrifuge at 3000 rpm at 4°C for 30 min.
8. Aspirate supernatant without disturbing the pellet.
9. Count precipitate with gamma counter. Calculate standard curve and unknown values.

Reagent Preparation:

All reagents are supplied in the kit.