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UC Davis - RadioImmuno Assay (RIA) Protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

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.

Materials

Reagents and Materials:

- Standards
- Test tubes
- Labeled antigen
- Antibody
- Precipitating solution
- Centrifuge

Prepare all standards and reagents according to kit instructions.

Before start

IMPORTANT: This is a generic RIA protocol. The steps may be slightly different for each assay. Refer to kit instructions for each specific kit for correct volumes, and incubation times.

1 Follow kit instructions for preparing standards and all reagents.

2 Follow kit instructions for volumes and incubation times. Pipet standards and samples . Pipet antibody and tracer. Incubate.

3 Follow kit instructions for volumes and incubation times. Add precipitating reagent. Incubate.

4 Centrifuge.

5 Aspirate supernatant.

6 Count precipitate with gamma counter.