Low-binding plate for inhibition step

Dilute samples and prepare titration of standards

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Mix the sample/standards with a constant amount of a labeled antibody

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Incubate until antigen-antibody binding equilibrium is reached

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Transfer to coated and blocked high-binding plate →

High-binding plate for ELISA

Coat wells with the antigen

↓

Block coated wells

↓

↓

Incubate to allow binding of antibody left free in the inhibition mixture to the antigen coated to wells

↓

Remove the inhibition mixture with the antibody bound to the antigen in the sample

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Add a reagent (streptavidin-enzyme for antibody labeled with biotin) to detect labeled antibody bound to the antigen coated to wells

↓

Add a chromogenic substrate for the enzyme

↓

Read color which is inversely proportional to the content of the antigen in the sample