



Principles of the

Elisa Technique

By

Maria da Costa

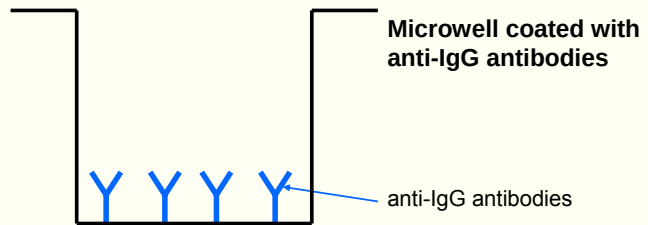


Competitive EIA





Competitive Elisa (1)



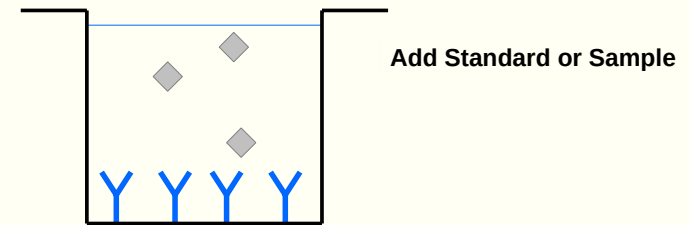
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Competitive Elisa (2)



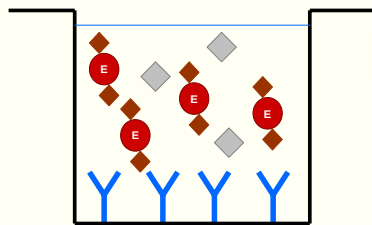
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Competitive Elisa (3)



Add Enzyme Conjugate

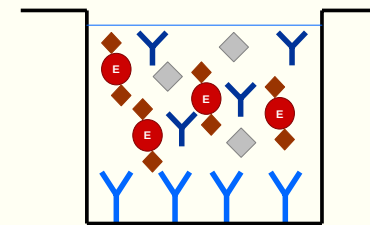


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Competitive Elisa (4)



Add Antibody Solution

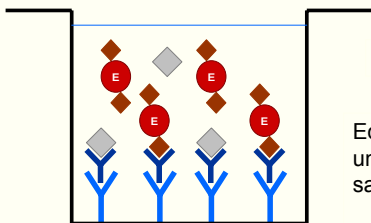


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Competitive Elisa (5)



Reaction starts

Equilibrium between bound and unbound analyte of standards or samples and enzyme-conjugated

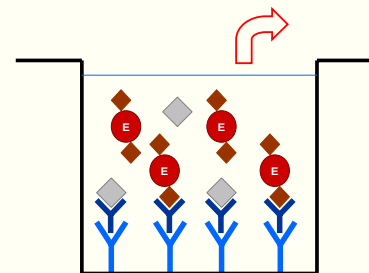


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Competitive Elisa (6)



Wash 3x

Removal of excessive reagents

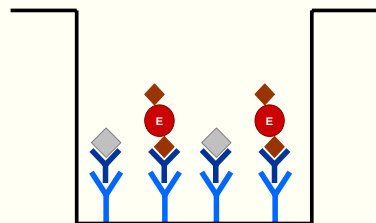


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Competitive Elisa (6)



After 3x Washing

Total removing of unbound reagents

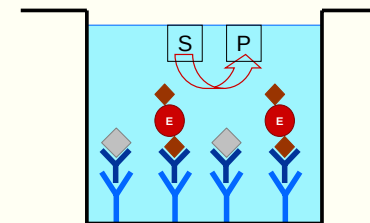


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Competitive Elisa (7)



Add Substrate/Chromogen

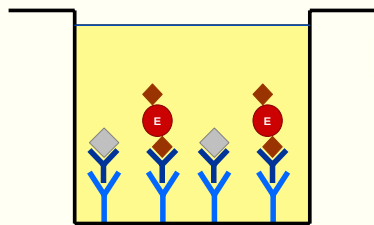
Substrate (S) develops a colored Product (P) in the presence of enzyme



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Competitive Elisa (8)



Add Stop Solution

Reaction is stopped and the developed color is measured visually or by means of a spectrophotometer

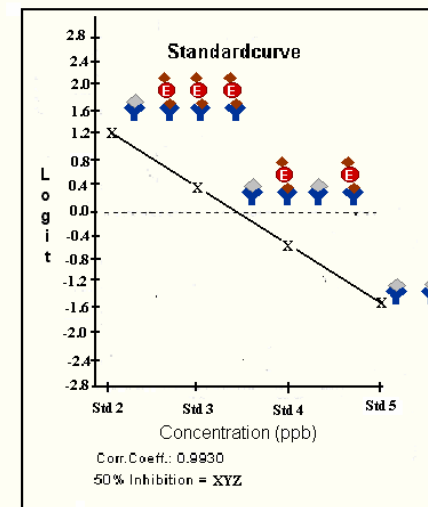
The intensity of the color inversely proportional to the concentration of the sample/standard - the more color, the lower the concentration in the sample/standard



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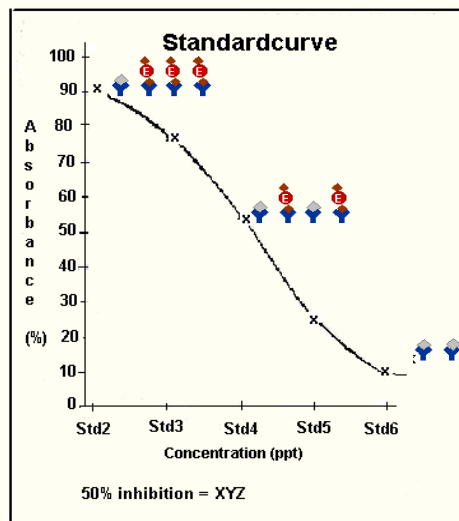
FAST test standard curve



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Long test standard curve



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Sandwich EIA

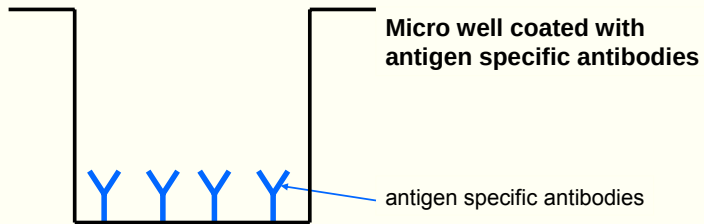
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Sandwich Elisa (1)



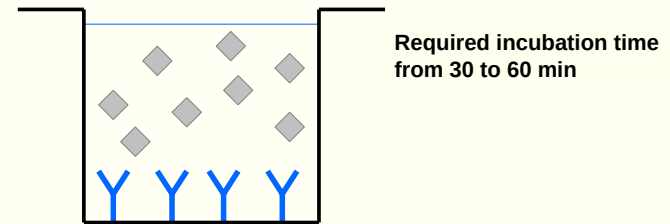
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Sandwich Elisa (2)



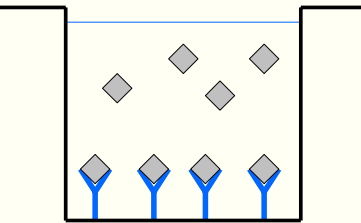
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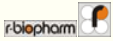


Sandwich Elisa (3)



**Required incubation time
from 30 to 60 min**

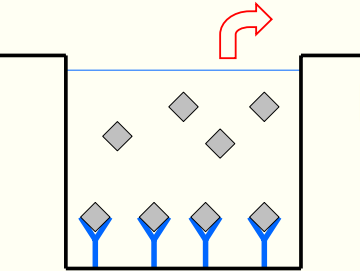
There will be an equilibrium of
bound and unbound antigen



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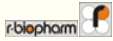


Sandwich Elisa (3)



Wash 3x

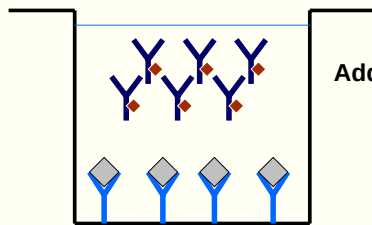
Removal of unbound antigen of
standard or sample



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Sandwich Elisa (4)



Add antibody-enzyme conjugate

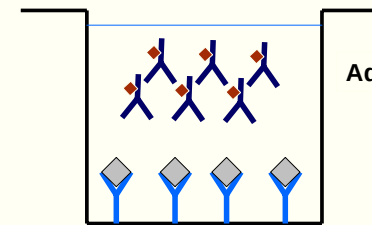


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Sandwich Elisa (4)



Add antibody-enzyme conjugate

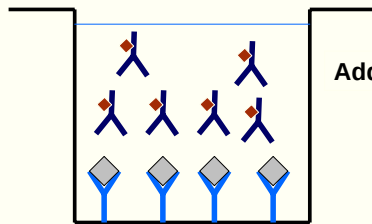


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Sandwich Elisa (4)



Add antibody-enzyme conjugate

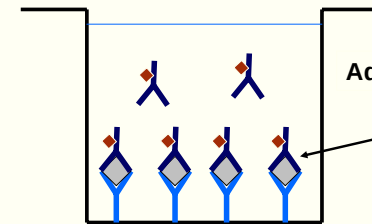


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Sandwich Elisa (4)



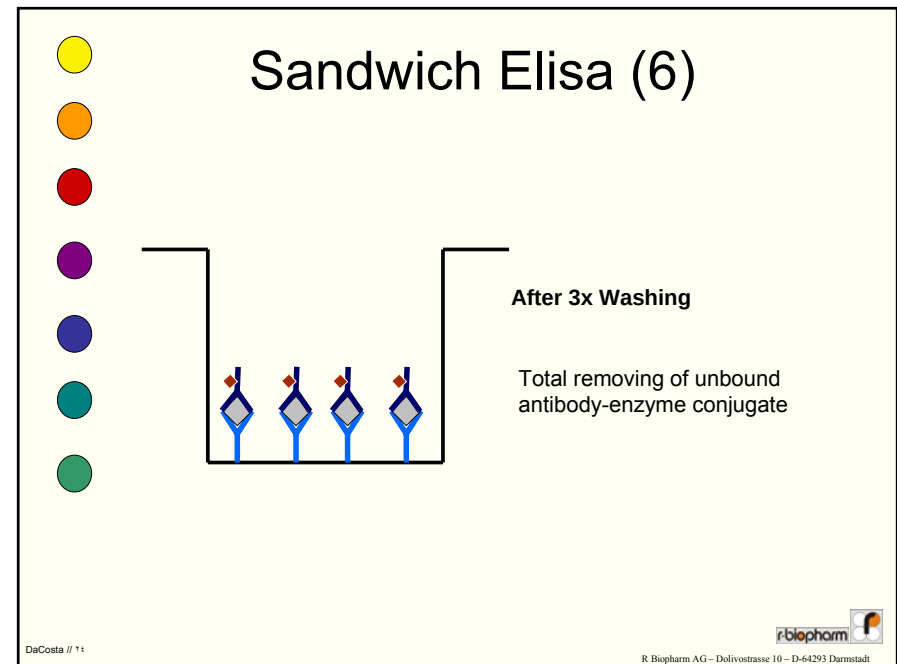
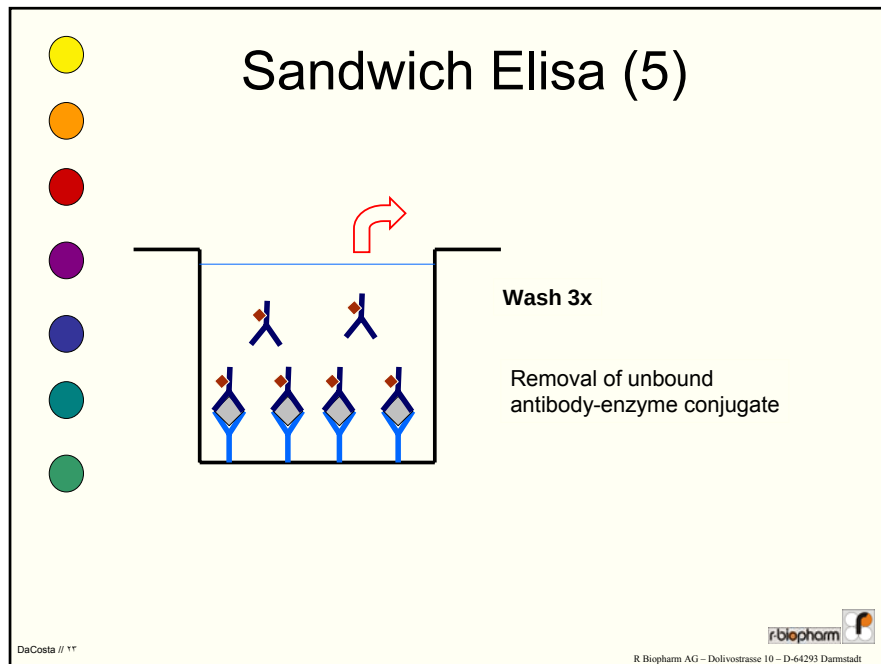
Add antibody-enzyme conjugate

There will be a binding
to the antigen-antibody complex

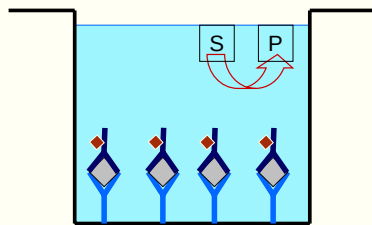


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Sandwich Elisa (7)



Add Substrate/Chromogen

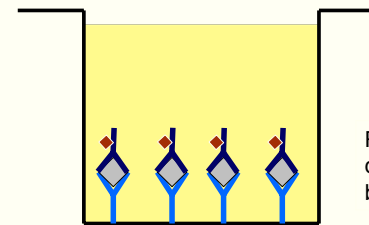
Substrate (S) develops a colored Product (P) in the presence of enzyme

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Sandwich Elisa (8)



Add Stop Solution

Reaction is stopped and the developed color is measured by means of a spectrophotometer

The intensity of the color is directly proportional to the concentration of the sample/standard - the more color, the higher is the concentration in the sample/standard

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Sandwich standard curve

