E. Hyatt R Obst/ 06

ELISA Protocol

Wash Buffer:

	PBS
0.1%	Tween-20

Blocking Buffer:

	PBS
0.1%	Tween-20
3%	BSA

AP Base Buffer:

96ml	di-Ethanolamine
1180μl	2M $MgCl_2$
800ml	H_2O

1. Coat antigen (5μg/ml in PBS) in 150μl in Costar EIA/RIA flat bottom 96 well plate (No. 9017).

2. Cover the plate and incubate @ 4C overnight.

3. Decant by flicking the plate and incubate 30mins. w/ 150μl blocking buffer @ 37C. Wash 3x w/ wash buffer.

4. Fill the wells w/ 100μl of serum samples diluted in wash buffer (start w/ 1:10), cover and incubate 1h @ RT.

5. Flick the plate and wash 3x w/ wash buffer.

6. Fill the well w/ 100μl anti-mouse IgG-AP (1/1000-1/2500 in wash buffer), cover, and incubate 1h @ RT.

7. Flick the plate and wash 3x w/ wash buffer.

8. Prepare substrate solution by dissolving one Phosphatase Substrate Tablet (Sigma No. 104-105) in 5ml AP Base Buffer (=> 1mg/ml).

9. Fill the wells w/ 100μl of substrate solution and read the color development in a microplate reader @405nm. The reaction can be stopped by adding 100μl 1M NaOH and preserved covered @ -20C.