

ELISA***Xenopus*****F 3 (06/14/15)**

- 1) Adsorb overnight at 4 C. (100 μ l/well) with FV3 stock diluted to 0.5 to 1×10^7 PFU in mammalian PBS pH 8.0 in 96-well plate, or with heat-killed .
 - For negative controls, adsorb 100 μ l (1:100 dilution) of normal the cell lysate used to generate high titer of FV3 (BHK or A6) and another with PBS containing 1% BSA.
 - For positive controls:
 - (a) Adsorb 10-100x diluted alloserum that contains a good titer of Ig to control the binding of the -specific secondary mAbs
 - (b) Adsorb directly 11D5 or 10A9 or any other mouse IgG isotype, to control the binding and signal of the tertiary HRP-conjugated anti mouse Ab.

- Prepare all samples in triplicate -
- 2) Wash and block 3x 10 min at RT with 200 μ l/well of Blocking Buffer (PBS + 1% BSA)
- 3) Add 100 μ l/well of serum dilutions from immunized and naïve animals. (for virus use 1:50 to 1:200 dilutions in PBS in triplicate, for bacteria use 1:100 to 1:1,000 dilutions in PBS in triplicate). Incubate 1-3 hrs at RT., or overnight at 4C.
- 4) Wash 3x 10 min with wash buffer
- 5) Add 100 μ l/well of 2x diluted 11D5 supernatant (for measurement of IgY) or 10A9 (for IgM) and incubate for 2 h at RT or overnight at 4C.
- 6) Wash 5x 10 min with wash buffer
- 7) Add 100 μ l/well of Rabbit anti-Mouse IgG-HRP (Sigma A-9044) diluted 1:5000 in blocking buffer. Incubate for 1 h at RT.
- 8) Wash **6** **8** x
- 9) Develop with 100 μ l of 1 Step Ultra TMB (Pierce, #34028) 30 – 60 min at RT.