ELISA for detection of *Xenopus* antibodies against FV3 (06/14/15)

1) Adsorb overnight at 4°C. (100 µl/well) with FV3 stock diluted to 0.5 to 1x10^7 PFU in mammalian PBS pH 8.0 in 96-well plate, or with heat-killed *E. coli*.
   - For negative controls, adsorb 100 µl (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 *Xenopus* alloserum that contains a good titer of Ig to control the binding of the *Xenopus*-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 µl/well of Blocking Buffer (PBS + 1% BSA)

3) Add 100 µl/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 µl/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 µl/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 to 8** x

9) Develop with 100 µl of 1 Step Ultra TMB (Pierce, #34028) 30 – 60 min at RT.

10) Block the reaction with 100 µl of 1M H_2SO_4 and read the plates at 420nm.

SOLUTIONS:

PBS: Use mammalian PBS (Sigma #3744)

Wash Buffer: PBS + 0.05% Tween-20 (1% BSA can be added to increase stringency)

Blocking Buffer: PBS with 1% BSA

Heat-killed bacteria: *E. coli* (XL1-blue, Stratagen La Jolla CA) cultured overnight at 37C, are boiled 30 min, spun and resuspended in 0.1 volume of PBS