

**ELISA for detection of *Xenopus* antibodies against FV3
(07/08/03)**

Adsorb 100 µl/well of FV3 stock diluted 1:100 in mammalian PBS in 96-well plate overnight at 4°C. for negative controls, adsorb 100 µl of normal A6 lysate (1:100) or PBS containing 1% BSA). Prepare wells in triplicate.

Wash 3x with 200 µl/well of Wash Buffer (PBS + 0.05% Tween-20).

Block with 100 µl of Blocking Buffer (PBS with 1% BSA) for 1 h at RT.

Wash 3x with 200 µl/well of Wash Buffer.

Add 100 µl/well of serum dilutions from immunized and naïve animals. (use 1:50 1:100 and 1:200 dilutions in PBS in triplicate). Incubate 1 h at RT.

Wash 3x.

Add 200 µl/well of 11D5 supernatant (for measurement of IgY) or 10A9 (for IgM) and incubate for 1 h at RT.

Wash 3x.

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.

Wash **6**x

Add 100 µl/well of ATBS solution (Pierce 1-step ATBS #37615). Incubate 30 – 60 min at RT.

Read plate at 405 nm.

SOLUTIONS:

PBS: Use mammalian PBS (Sigma 3744)

Wash Buffer: PBS + 0.05% Tween-20

Blocking Buffer: PBS with 1% BSA