Protocol: Vitellogenin protein estimation by ELISA

Day one

1. Coat EIA plate

- Dilute test samples to a concentration of 1 µg-3 µg per 90 µl 1X PBS. 90 µl of samples is coated onto one well of 96-well microtiter EIA plate, usually I perform a duplicate for each test sample.
- Stand the plate at plate at room temperature (RT) over night (O/N).

Day Two

1. Blocking the plate

- Discard coated protein samples, Tap the plate against paper towel to remove as much liquid as possible.
- Add 315 µl of EIA blocking buffer (BSA freshly added to a concentration of 1g/L) using a multichannel pipette and a cell culture reservoir.
- Shake the plate for 2-3 hr at RT.

2. Wash

- Discard EIA blocking buffer, tap plate.
-Rinse plate two times with 200 µl-300 µl PBS-Tween. Shake 5 minutes using orbital shaker after adding PBS-Tween.

3. Primary antibody

- Dilute primary antibody (anti-TcVg1 multiclonal antibody made in rabbit) 1: 1500 in EIA-BSA buffer (BSA freshly added to a concentration of 1g/L).
- Add 100 µl of primary antibody to each well except for blank well (Blank well contains only PBS without any test samples).
- Shake 2 minute. Then incubate at RT for 1-2 hr.

4. Wash

- Discard primary antibody, tap plate.
-Rinse plate three times with 200 µl-300 µl PBS-Tween and shake 5 minutes.

5. Secondary antibody

- Dilute secondary antibody (anti-rabbit IgG horse reddish peroxidase linked antibody from donkey) 1: 2500 in EIA-BSA buffer (BSA freshly added to a concentration of 1g/L).
- Add 100 µl of primary antibody to each well.
- Shake 2 minute. Then incubate at RT for 1 hr.

6. Wash

- Discard secondary antibody, tap plate.
-Rinse plate three times with 200 µl-300 µl PBS-Tween and shake 5 minutes.

7. Setup enzyme reaction

- Add 100 µl of substrate TMB to each well.
- Wait for 10-15 minutes at RT (You will see the blue color showing up).
- Add 100 µl of 1M phosphoric acid to stop the enzyme reaction and blue color turns into yellow.
- Read plate at 450 nm using a plate reader. Absorbance of each sample (Blank reading is subtracted from each sample) is used from data analysis.

Note: If necessary, test each coated sample with and without adding primary antibody (secondary antibody added for both).Then absorbance for well with no primary antibody is subtracted from well containing primary antibody to eliminate the effects of non-specific binding to secondary antibody.