Protocol to quantify BKD antigen p57

Protocol is outlined in the "Renibacterium salmoninarum antigen test kit" –paper by DiagXotics Inc. and in Pirhonen, Schreck & Gannam (2000), Appetite of chinook salmon naturally infected with bacterial kidney disease, Aquaculture 189: 1-10.

If antibodies are used separately instead of using the test kit following suggestions and advice might be helpful.

Sample preparation:

I homogenized samples with the buffer by using a pellet pestle mixer in 1.5 – 2 ml eppendorf tubes. I reused pestles after washing them after each sample in 90% ethanol and distilled water. Sample sizes up to 0.3 – 0.4 g can be homogenized in these tubes, otherwise transfer the sample for larger culture tubes for homogenizing. To avoid this transfer you should take the sample size into account when sampling the kidneys. On the other hand sample is more representative if the whole kidney is taken from each fish.

Sample dilution buffer was as follows:

PBS (8.9 g/l NaCl, 6.7 g/l Na_2HPO_4, 0.2 g/l NaH_2PO_4; pH should be 7.8 – 8.2) + 0.5% (w/v) BSA (I used "flakes").

After maceration I centrifuged samples for 7 min at 6000 RPM by using Becman GS-15R centrifuge with F3602 rotor.

Analysis:

Coating buffer was as a carbonate-bicarbonate solution:

Na_2CO_3 1.59g/l, $NaHCO_3$ 2.93 g/l and NaN_3 (=sodium azide) 0.20 g/l. Adjust pH to 9.6 (this is critical!) and store at 4°C no more than two weeks. Before coating (overnight at 4°C) add monoclonal antibody to the buffer, for me it worked at about 3 ug/ml concentration, from diagxotics 2 ug/ml was suggested. I added 50 ul of this buffer into each well. The plates I used were Corning disposable sterile ELISA plates (25805-96).

The following morning just strike the plate dry against a towel, and add the blocking solution (appendix 1), 100 ul in each well. Incubate at room temperature for 1 hour and strike the plate dry against a towel.

After this continue from the step five as described in the protocol paper.

Instruction to make Streptavidin-HRP is shown in the appendix 3. You can make a stock solution of PBS+BSA and add STP-HRP separately for a smaller amount of buffer every day, and no filtration is necessary.

Instructions to make ABTS solution is shown in the appendix 4.

Adding stop buffer is not necessary , but for me it worked (2% SDS) giving more accurate values for replicates. I red the plates at 405 nm.

Washing solution was PBS + 0.5% Tween 20, and the platewasher (Ultrawash plus) was programmed as 8-4-4-3. After each washing remember to strike the plate dry against a towel.

Appendix 1.

Blocking solution

Make in three steps:

1. Tris buffer saline (TBS)

	g/l	g/200ml
Trizma Base	6.07	1.214
EDTA	0.37	0.074
NaCl	8.7	1.74

Adjust pH to 8.0

2. Add Tween 20 (1% v/v) in TBS (T-TBS)
3. Add BSA (3% w/v; I used “flakes) in T-TBS