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Supplementary materials

Development of a Highly Sensitive Real Time Immuno-PCR for the Measurement of Chloramphenicol in Milk Based on Magnetic Beads Capturing

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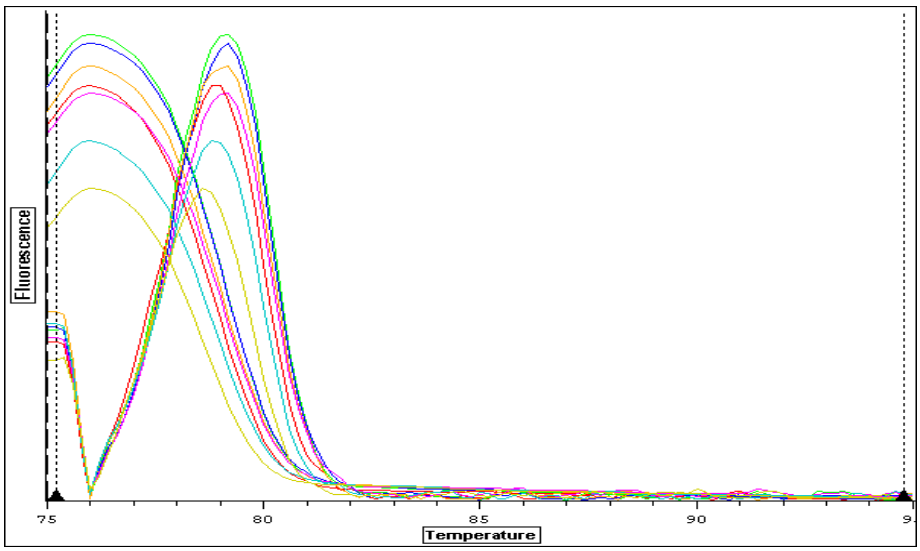

Supplementary Figure 1 Melting curve analysis of RT-IPCR for CAP

Procedure of direct competitive CL-ELISA

Plates were coated overnight at 4 °C with 100 μL of CAP-OVA dissolved in buffer a (1.5 μg/mL). The plates were washed with 260 μL/well buffer c manually three times, blocked with 200 μL/well of buffer b and incubated at 37 °C for 1 hour. After the plates were washed as described above, then 100 μL/well of mixture of DNA reporters (1.5 μg/mL) and HRP-conjugated anti-CAP MAb (1/5000 dilution) (scheme A) or 100 μL/well of HRP-conjugated anti-CAP MAb (1/5000 dilution)(scheme B) in buffer d were added, respectively. The competitive reaction was allowed to take place for 30 min at room temperature. After washing five times and finally the HRP tracer activity was revealed by adding 100 μL/well of a freshly prepared substrate mixture of SuperSignal substrate solution. The intensity of light emission was measured at 425

29 nm using a chemiluminesence reader immediately after the addition of the substrate
30 and the results were expressed in relative light units (RLU). Eventually, the RLU for
31 scheme A was equal to that for scheme B when the concentration of anti-CAP was 0,
32 proving that the DNA reporters in scheme A did not bind with anti-CAP.