**Supplementary information**

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![Figure 1](image_url)

**Figure 1.** Selection of target events to be monitored by gating and bead-map region selection
Figure 2. Histogram plot of fluorescence intensity of different calibration points of melamine standard curve
**Figure 3.** Melamine calibration curve obtained with three batches of microspheres immobilized with 10, 50 or 100µg of AETT. All data points are expressed as the percentage inhibition in the absence of any melamine (mean ± SEM, n=2)

**Figure 4.** Melamine calibration curve obtained with three different competition formats. For one-step incubation (blue curve), the total incubation time was 1 hour. For two-steps incubation (red and green curves), each step was incubated for 30 min. All data points are expressed as the percentage inhibition in the absence of any melamine (mean ± SEM, n=2)
**Figure 5.** Melamine calibration curve obtained with five different incubation times. All data points are expressed as the percentage inhibition in the absence of any melamine (mean ± SEM, n=2)

**Figure 6.** Optimization of 1st antibody concentration. Each data point was obtained under different concentrations of 1st antibody in the absence of melamine. The signals were normalized to the highest signal measured (5800ng/ml) and expressed as normalized fluorescence intensity (mean ± SEM, n=2)
Figure 7. Optimization of 2\textsuperscript{nd} antibody concentration. Each data point was obtained under different concentrations of 2\textsuperscript{nd} antibody in the absence of melamine. The signals were normalized to the highest signal measured (3000ng/ml) and expressed as normalized fluorescence intensity (mean $\pm$ SEM, n=2)