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#### **ACCEPTED MANUSCRIPT**

# Digital analysis with droplet-based microfluidic for the ultrasensitive detection of $\beta$ -Gal and AFP

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#### **Abstract**

In this study, we presented a digital analysis with droplet-based microfluidic, which was applied for the detection of  $\beta$ -galactosidase ( $\beta$ -Gal) and AFP. The  $\beta$ -Gal was quantified according to the Poisson distribution in digital analysis with droplets containing  $\beta$ -Gal and fluorogenic substrate fluorescein di- $\beta$ -D-galactopyranoside (FDG). In our method, the lowest concentration of  $\beta$ -Gal we could accurately detect was 5 fM. We found that the digital detection could be applied in quantifying protein biomarkers, take AFP for model sample. The AFP was detected through indirect detection with excess streptavidin-conjugated  $\beta$ -galactosidase (SA- $\beta$ -gal) as signal tag. To be specific, firstly SA- $\beta$ -gal was conjugated to biotin-labeled AFP antibody, and then the unbounded signal tag in the reaction solution was taken out for digital detection with droplet-based microfluidic. As a result, the AFP could be also

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