Direct competitive ELISA protocol

A. Theory：

By the specificity of different antibodies to antigens, to detect the materials in the sample.

B. Materials：

- ELISA plate (high binging, strip)
- 8 channel pipette
- 0.01M PBS
- PBS-BSA
- Sal-SH-EDC/NHS-HRP conjugates
- 3,3’5,5’ tetramethylbenzidine(TMB)
- 1N HCl

C. Laboratory procedures：

1. Coating:
    - Add 100 μl of 500-fold diluted anti-Sal polyclonal antibody into every wells and incubate at 37℃ for 1 hour.
    - After incubation, wash the plate 4 times with 0.01 M PBS containing 0.05% Tween 20.
2. Blocking:
    - 170 μl of 0.1% BSA in 0.01 M PBS is added into every wells and incubate at 37℃ for 30 min.
    - After incubation, wash the plate 4 times with 0.01 M PBS containing 0.05% Tween 20.
3. Competition:
    - 50 μl of Sal standards(500, 50, 10, 1, 0.1 and 0.01 ng/ml) and 50 μl of 4000-fold diluted Sal-SH-EDC/NHS-HRP are added into wells, then mixed well and incubated at 37℃ for 1 hour.
    - After incubation, wash the plate 4 times with 0.01 M PBS containing 0.05% Tween 20.
4. Colorimetric:
    - Add 100 μl 3,3’5,5’ tetramethylbenzidine(TMB) into every wells and keep in dark for 20-30min.
5. Stop reaction:
    - After colorimetric, add 100 μl 1N HCl into every wells for stop reaction. Detect absorbance of 450nm/650nm.