Experimental Scheme of Carboxylated Latex Conjugated Antibody

(1) Solution formulation

- Basic buffer: 0.05M boric acid buffer solution pH 7.0, pH 7.5, pH 8.0

Liquid A: 0.2mol/L boric acid 0.05mol/L NaCl

Formula: Boric acid: 0.6184g, NaCl 0.1462g with distilled water to 50ml

Liquid B: 0.05 mol/L sodium borate

Formula: 0.9535g sodium borate with distilled water to 50 ml

PH	A(ml)	B(ml)
7.09	9.4	0.6
7.60	8.5	1.5
7.94	7.5	2.5
8.08	7.0	3.0

- 10 times concentrated sealing liquid

Raw Material	10mL
0.5M Tris-HCL Buffer	10ml
Tween-20	0.1ml
BSA	1g

- . Coupled storage solution (0.05M boric acid buffer solution (pH8.0))

Raw Material	10mL
0.5M Boric Acid Buffer Solution	10ml
Tween-20	0.05ml
BSA	0.02g

(2) Coupling process

- Take 0.05M/pH 7.0 boric acid buffer solution 100ul into 1.5ml centrifugal tube, add 100ul no-load latex, whirlpool oscillation, mix and clean.
- 0.05M/pH 7.0 boric acid buffer solution re-sol latex, adding EDC, NHS for activation.
- 20000r, 10 C, 20 min centrifugation, discarding supernatant, using 0.25ml 0.05M/pH 7.5 boric acid buffer solution resolving (centrifugal time can be prolonged or shortened according to centrifugal effect, the same below). Wash 2-3 times with coupling buffer 0.25ml 0.05M/pH 8.0 boric acid buffer.
- Ultrasound dispersion, 100W, 1min, 3s, 3S (if there is a particle size analyzer, check particle size and dispersion coefficient PDI≤ 0.07). The final concentration should

be 20-200 ug/ml after adding coupling protein PCT. Put it in 250R, 20℃ shaker for 2 hours.

- The final concentration of BSA was 1% by adding both Liquid A and B 28ul 10 times of concentrated sealing liquid and keep sealed in shaker. (1-2 h).
- centrifugal washing 2-3 times, 20000R, 10℃, 20min, discarding supernatant, resolving with 0.25ml 0.05M/pH 8.0 boric acid buffer; after the last washing and centrifugation, resolving with coupling storage buffer to 50ml, ultrasonic dispersion, 50w-200w, 1min, 3s, 3S (if there is a particle size analyzer, check particle size and dispersion coefficient PDI≤ 0.07). Store at 4 C.