Title:	**SOP:** Covalent coupling of aSARS to NP
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Protocol number:	FZS084, FZS080, FZS081

1 MATERIALS

- 0.1 % Particles 300 nm (Eu, QD, Blue, Green, Orange, Yellow)
- deionized water (DI water)
- PEG 20k
- Tween20
- anti-SARS 302 2 g/L (provided by FZMB)
- EDC
- S-NHS
- MES buffer
- Sodium acetate buffer (NaAc)
- PBS
- Ethanolamine
- Sodium azide (azide)

Required solutions:

- Activation buffer (AB): 5 mM MES pH 5.5; 1 mM azide*
- Reaction buffer (RB): 5 mM NaAc pH 5.5; 0.5 % PEG 20k; 1 mM azide*
- Wash buffer (WB): 10 mM Hepes pH 7.4; 0.5 % PEG 20k; 0.01 % Tween20; 1 mM azide*
- 15 % PEG 20K
- 0.3 % Tween 20
- 10 mg/mL EDC in AB 5 mM MES (use immediately!)
- 10 mg/mL S-NHS in AB 5 mM MES (use immediately!)
- 25 % / 4 M Ethanolamine in DI water (use immediately!)

*The Sodium azide is an optional component and is used for storing the buffer solutions in the fridge.

2 PARTICLE ACTIVATION

- 250 µL particle suspension (0.1 %)
- centrifuge for 3-5 min at 12400xg **; discard supernatant
- add 235 µL AP, resuspend carefully and use Ultrasonic bath 10-30 s ***
- add 5 µL EDC and 10 µL S-NHS (c 10 mL/mL in AP)
- incubate 30 min at room temperature on a shaker 1600 rpm
- centrifuge for 3-5 min 12400xg ** and discard supernatant

3 COVALENT COUPLING

- resuspend the pellet carefully in 0,25 mL RB, use ultrasonic bath 100% 10-30 s, if necessary ***
- add 3.13-6.25 µL of antibody (concentration 2 g/L, 3.13 µL for research, 6.25 µL for customers and QD)
- incubate for 60 min at room temperature on a shaker 1600 rpm
- add 5 µL of 25 % Ethanolamine, 5 µL 15 % PEG 20K and 5 µL 0.3 % Tween20 to the mix
- incubate for an additional 30 min at room temperature on a shaker 1600 rpm
- centrifuge for 3-5 min at 12400g **; discard supernatant
- wash twice with 250 µL WB, use ultrasonic bath 100% 30 s, if necessary ***
- resuspend in 250 µL WB (final concentration theor. 0.1 %)
- in the case of large losses due to washing, take up in less WB (e.g. 200 µL) and determine the concentration by UV/Vis (10 µL+190 µL MilliQ in a micro cuvette)

** Suggested centrifugation speed for Batch PSF007_52, it can be further optimized to reduce agglomeration. Note: Centrifugation speed might change after activation and coupling.
*** Always check if it is at room temperature otherwise the Particles or the antibody can be damaged, ultrasonic bath is mostly optional but can improve performance

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