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— Replicate freeze-activated SPAN (2nd repetition)

① Modify NH_2 -APT with OBO-NHS.

1 nmol $\rightarrow$ 2 columns (0.5 nmol). (1 for sample and 1 for calib).

in OBA-low binding vial - 5 eq

$\rightarrow$ 1 nmol apt all together for modif: 5 nmol OBO-NHS (2.48 μg).

dol in PBS pH 8.3 : 0.26 mg/ml

$$\rightarrow 266 \mu\text{g} \cdot 1000 \mu\text{l} = 2.48 \mu\text{g} \cdot x$$

$$x = 9.3 \mu\text{l}$$

Tot: 20 μl (19.3)
 Stir 2h, rt

② After 2h. Add PBS pH 9.5 (20 μl) + 5 other eq of OBO-NHS (9.3 μl)

(2h, rt) (TOT: 47.9 μl)

③ Remove excess OBO

$\rightarrow$ Divide in 2 col (0.5 nmol apt each): 23.95 μl each?

— Resusp. in water while vortexing

— loose cap 1/4 and twist off cap

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