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- Attempt at QUANTI of avidin: using biotin-5-~~fluor~~ conjugate

- Tried with older fluorophore → NOT conclusive.

- Re ordered new (1 mg) → Aliquotes: 5 mg/ml → resuspension in 200 μ l total Omp → aliq. 20 μ l each.

Protocol:

Method for avidin quantitation based on the theory that avidin can quench fluo of biotin(5-fluor) conjugate.

Briefly, 70 μ l of 1 mg/ml ^{in previous paper is 1.0 μ g/ml (1/1000)} biotin-5-fluor in 0.1x PBS buffer (pH 7.2) was added in certain vbe (see append on f).

~~Various volumes of 10 μ g/ml avidin in 0.1x PBS (making up from 0 to 1 μ g) and 0.1x PBS were added to keep final volume of 1 ml in each solution. Each sol was incubated for 30 min in the dark. Fluorescence of the sol. was measured (lex/lem = 490/523 nm) in the spectrofluorometer.~~

12 standards:

- | | | | 10 μ g/ml | 1 μ g/ml |
|----|--------------------------------|------------------|------------------|-----------------|
| 35 | (A) 0 μ g/ml → 0 " → | 0 μ l New + | 70 μ l Bio + | 930 μ l PBS |
| | (B) 0.05 μ g/ml → 0.05 " → | 5 μ l New + | 70 μ l Bio + | 925 μ l PBS |
| | (C) 0.1 μ g/ml → 0.1 " → | 10 μ l New + | 70 μ l Bio + | 920 μ l PBS |
| 40 | (D) 0.2 μ g/ml → 0.2 " → | 20 μ l New + | 70 μ l Bio + | 910 μ l PBS |
| | (E) 0.3 μ g/ml → 0.3 " → | 30 μ l New + | 70 μ l Bio + | 900 μ l PBS |
| 45 | (F) 0.4 μ g/ml → 0.4 " → | 40 μ l New + | 70 μ l Bio + | 890 μ l PBS |
| | (G) 0.5 μ g/ml → 0.5 " → | 50 μ l New + | 70 μ l Bio + | 880 μ l PBS |
| | (H) 0.6 μ g/ml → 0.6 " → | 60 μ l New + | 70 μ l Bio + | 870 μ l PBS |
| | (I) 0.7 μ g/ml → 0.7 " → | 70 μ l New + | 70 μ l Bio + | 860 μ l PBS |
| | (J) 0.8 μ g/ml → 0.8 " → | 80 μ l New + | 70 μ l Bio + | 850 μ l PBS |

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