***Prepare standards***

*Break ampule in hood. Prepare dilutions in 1.5 ml tubes.*

|  |  |  |  |
| --- | --- | --- | --- |
| **Vial** | **μl KHPO** | **μl BSA** | **μg / ml** |
| A | 0 | 300 stock | 2000 |
| B | 125 | 375 stock | 1500 |
| C | 325 | 325 stock | 1000 |
| D | 175 | 175 vial B | 750 |
| E | 325 | 325 vial C | 500 |
| F | 325 | 325 vial E | 250 |
| G | 325 | 325 vial F | 125 |
| H | 400 | 100 vial G | 25 |
| I | 400 | 0 | 0 |

***Prepare working solution (WS)***

n = # experimental samples (include replicates)

(n + 9) \* 2 = \_\_\_\_\_\_\_ml WS needed

**Working solution mix**

Reagent A : (n + 9) \* 2 = \_\_\_\_\_\_\_ml Reagent A

Reagent B: ml Reagent A/50 =\_\_\_\_\_ml Reagent B

***Incubate samples***

In glass test tubes, mix 100 μl each sample/standard with 2 ml WS. Mix thoroughly.

Cover with foil and incubate at 37°C for 30 minutes.

Cool tubes to RT.

***Spec samples***

*Spec all samples within 10 minutes of each other.*

Set spec to 562 nm. Label n+9 plastic cuvettes. Blank with water.

Read each sample for 3 technical replicate measurements.