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Qubit (HS dsDNA Assay) V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

Qubit (HS dsDNA Assay)



Prepare working solution

5m


1


NOTE: This is suitable for small numbers of samples (i.e. less than 24), including the quantification of the final libraries using the next generation sequencing SOP.

Label the required number of 0.5 ml tubes for Standard 1, Standard 2 and all your samples (use only the specific Qubit Assay tubes).

Label a 5 ml tube with 'Qubit Working Solution'.

2

Add  199 $\mu\text{L} \times n$ Qubit Buffer (either BR or HS, depending on the assay you want to do) to the 5 ml tube ($n = \text{samples} + 2 \text{ standards} + 1$).


Add  1 $\mu\text{L} \times n$ Qubit Reagent (either BR or HS, depending on the assay you want to do) to the tube, mix by vortexing.


Tip: use a low vortex speed to avoid frothing; excessive frothing may require the preparation of additional diluted reagent to allow sufficient volume for all samples

Add samples to tubes and incubate


4m


3

Alliquot  190 μL Qubit Working Solution into each of the tubes used for Standard 1 and Standard 2.


Alliquot  198 μL Qubit Working Solution into each individual assay tubes

4

Add  10 μL of Qubit Standard 1 and Standard 2 to the appropriate tube (the final volume will be 200 μL). Mix by vortexing 2-3 seconds, be careful not to create bubbles.

Add  2 μL stock DNA to the tube (the final volume will be 200 μL). Mix by vortexing 2-3 seconds, be careful not to create bubbles.

5

Incubate all tubes at room temperature for  00:05:00 .

Tip: the fluorescence signal is stable for 3 hours at room temperature, so samples can be re-read if necessary

5m

Read standards

6

Select high-sensitivity double-stranded DNA assay on the Qubit and read the standards when prompted

Read samples



- 7 Read samples when prompted (no need to re-read standards between readings) and record DNA concentration in **ng/ul**.