

## Protocol for PicoGreen quantitation of Tube DNA Samples

- 1) Spin down tubes and array on a 96-position tube rack, leaving the bottom row empty.
- 2) In a 96-well plate, add 80 uL of TE (provided in Pico Kit) to the first 11 columns.
- 3) Using the 10 uL Multichannel pipettor's dilute function, draw up 6 uL TE, then 1.5 uL DNA from the tube.
- 4) Deposit the DNA in the corresponding well in the 96-well plate. –This is the dilution plate.
- 5) Repeat this step for all DNA sample tubes.
- 6) Using the 250 uL Multichannel Pipettor, add 100uL TE to each DNA-containing well and mix.
- 7) Spin plate down if any bubbles remain.
- 8) Appropriately label 3 flat-bottom reader plates.
- 9) Using the 250 uL Multichannel Pipettor, draw up 90 uL TE, then 10uL DNA from the dilution plate and deposit in the corresponding wells in the reader plate. Repeat this process for the remaining reader plates.
- 10) Prepare DNA concentration standards in 1.5mL tubes. First, add 8uL DNA (provided in the kit) to 392uL TE. This is the Working Stock. Add TE and Working stock volumes accordingly to the table for the concentration curve samples:

Concentration	500	250	125	75	50	25	5	0
TE	170	240	315	370	380	390	398	350
Working Stock	170	80	45	30	20	10	2	0

- 11) Add 100uL of the concentration curve samples into the last column of each reader plate—starting with 0 in A12 and increasing concentration going down the plate.
- 12) Prepare Pico mixture: 100uL thawed Pico Reagent into 30 mL TE. Mix well.
- 13) Pour Pico mixture into a reagent reservoir and dispense 100uL into each reader plate well. Incubate for 10 minutes away from light , then read.
- 14) The samples will be at a final dilution of 1:2500, and the concentration standard values are in ng/mL.