# Picogreen Protocol

* The protocol assumes one 96-well plate.

## Materials

* (1) Costar black 96-well plate
* (8) 1.5ml micro-cfg tubes
* (2) 15 or 50 ml falcon tubes (depends on volume 1x TE buffer neded)
* (1) Picogreen kit
  + Warm up the picogreen reagent before use

## Make 1x TE buffer

* **NOTE:** Sketching out a plate layout is highly encouraged before beginning analysis.
  + The plate will contain:
    - Standards
      * Dilution series of DNA standard
    - Unknowns
      * Provided nucleotide samples with unknown concentrations
    - Blanks
      * Accounts for background noise in unknowns (from humics and other contaminants)
* **NOTE:** This protocol assumes all standards, unknowns, and blanks are run in duplicate.
* To make 1x TE buffer from the 20x stock supplied in the kit:
  + 1:20 dilution
    - e.g., 2 ml of 20x TE buffer in 38 ml of nuclease-free water
* Calculate the total volume of TE buffer needed:
  + raw\_total\_volume = TE for standards + TE for sample dilutions + TE for picogreen reagent dilution
    - volume for all standards (ul):
      * 14 \* 99 + 1832.5 = 3218.5 ul
    - volume for all blanks (ul):
      * Number\_of\_blanks \* 199
    - volume for all unknowns (ul):
      * Number\_of\_unknowns \* 198
  + final\_total\_volume = raw\_total\_volume \* 1.1
    - **Note:** Produces 10% extra 1x TE buffer
  + For example:
    - 10 unknowns in duplicate + 1 blank in duplicate + 14 total standards would need 5.59 mL of raw\_total\_volume and 6.15 mL final\_total\_volume (with extra TE buffer).
* use a 50 mL falcon tube for making the 1x TE buffer

## Make standards

* Stock standard conc.: 100 ug/mL
* Working standard conc. needed: 2 ug/mL
  + 1:50 dilution in TE buffer
* Volume working standard needed (ul): 500
* Volume TE buffer needed (ul): 500 \* 49/50 = **490**
* Volume stock standard needed (ul): 500 \* 1/50 = **10**
* Make up standards, as specified in table below, in 1.5 mL Eppendorf tubes
  + **Note:** This will make 2 replicates of each standard (enough for 1 plate).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Final Conc. post-pico (ng/mL)** | \_\_\_ | **TE to add (uL)** | \_\_\_ | **2ug/mL working std to add (uL)** |
| 750 |  | 62.5 |  | 187.5 |
| 500 |  | 125 |  | 125 |
| 250 |  | 187.5 |  | 62.5 |
| 100 |  | 225 |  | 25 |
| 50 |  | 237.5 |  | 12.5 |
| 20 |  | 245 |  | 5 |
| 0 |  | 250 |  | 0 |

\* 'post-pico' means the final conc. after adding the Picogreen reagent.

* Add 100 uL of each standard to unique wells of a Costar black 96-well plate. The standard curve should be duplicated.

## Unknown & blank well reagent additions

* Add 99 uL of TE buffer to all wells on the Costar plate assigned as unknowns or blanks.
  + Multichannel pipettors and reagent resevoirs can be used for this step if many unknowns/blanks are in the layout.
* Add 100 uL of TE buffer to all blank wells.
  + The blanks will not have Picogreen reagent added.
* Add 1uL of the corresponding unknown to the assigned unknown well.

## Making and adding Picogreen Reagent

* Based on the number of unknowns to be run, calculate the volume of 1x Picogreen reagent that should be made (volume in ul):
  + total\_volume (uL): 110 \* (number\_of\_unknowns + number\_of\_standards)
    - **NOTE:** Remember the duplicates!
* Once that the total volume is determined, calculate dilution of the 200x Picogreen reagent:
  + Volume of 200x Picogreen needed (uL): total\_volume \* 1/200
  + Volume of TE buffer needed (uL): total\_volume \* 199/200
* For example:
  + If running 10 unknowns in duplicate (& 14 total standards), 2640 uL of 1x Picogreen should be made, using 13.2 uL of 200x Picogreen reagent diluted with 2626.8 uL of 1x TE buffer.
* Make up Picogreen reagent in a falcon tube (15 mL or 50 mL, depending on the amount needed) that is wrapped in aluminum foil (to prevent degradation of reagent).
* Once reagent is made up, transfer 100 uL of reagent to each well of the Costar plate to be analyzed, EXCEPT the wells to be used as blanks.
  + Again, the multichannel pipettor and reagent resevoirs can be useful in this step.
  + Make sure to pipet up and down to mix reagent with well contents.
* Place the Costar plate in the plate reader and incubate for 5 minutes
  + Alternatively, just stick the plate in a drawer.

## Running unknowns on plate reader

* While unknowns are incubating, set up analysis program on plate reader software:

1. Open Softmax Pro 6.3 software.
2. Then, click on: 'Protocols' tab => 'Protocol Manager' => 'Protocol library' => 'Nucleic Acids' => 'Picogreen assay'. This will open up an already created Pico protocol.
3. To make sure the plate reader is set-up to run a fluorescence assay, click on the 'Settings Icon', choose the 'FL' option, and make sure that excitement is set to 485nm and emission is set to 535 nm.
4. Modify plate set-up by scrolling to the page with the plate layout, and clicking on the small plate icon ('Template editor'). This will open up a screen that will allow you to add standards, unknowns, and blanks to the plate to be read.
   * **Note:** The dilution factor for the unknowns should be set to 200.
5. Once the plate is modified, you can save as a protocol file. You can also save the file as a datafile once the analysis is done.
6. To read plate, open the plate reader using the open/close button and place the plate into the reader. Note the orientation of the plate! Then press the read button.