PeptiGel® Protocol:

PicoGreen® Cell Viability Assay for PeptiGel® cultures

Before you begin, please note:

This protocol describes the assessment of cell viability using the PicoGreen® fluorescent DNA quantification assay.

It's recommended to use a positive displacement pipette (such as the Gilson piston pipette) to allow easy pipetting as these are viscous hydrogels. Use of an air displacement pipette could lead to the introduction of bubbles to your PeptiGel®.

This protocol has been written as a guide. Therefore, further assay optimization may need to be carried out for your cultures.

Preparation:

- Prepare a fresh pronase solution at a concentration of 10 mg/mL in water. Please note, 400 µL volume is required for 100 µL of sample.
- Prepare 2 x TE buffer by diluting 20 x TE buffer with HPLC-grade water.
- Prepare a stock standard solution of Lambda DNA to a final concentration of 2 µg/ml, by diluting 1:40 in 200 x TE buffer.
- Prepare standard concentrations of Lambda DNA for the generation of a standard curve.

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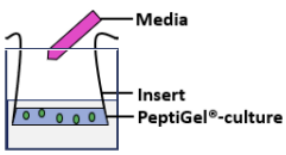
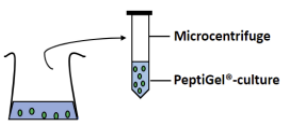
1. It is recommended to use this protocol with PeptiGels® made using the 3D Cell Culture method.

2. Carefully remove the cell culture medium from the PeptiGel® culture. An acellular PeptiGel® should be set up as a blank control.

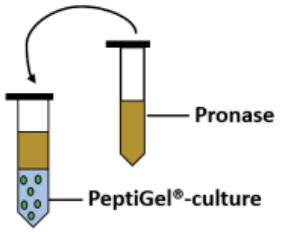


3. Remove the bottom membrane and transfer the PeptiGel® culture into a microcentrifuge tube.

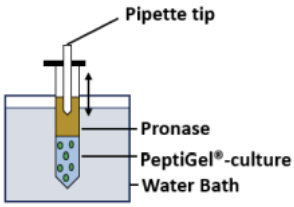


4. Agitate by tapping the tube gently until the PeptiGel® culture falls to the bottom of the tube.

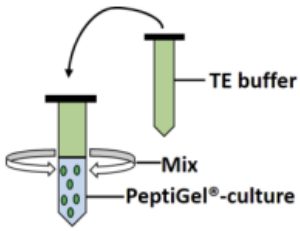
5. Add 400 µL of pronase solution to the tube and mix by pipetting gently.



6. Incubate the tube in a water bath at 37°C for 5 minutes, agitating every minute by carefully shaking the tube.

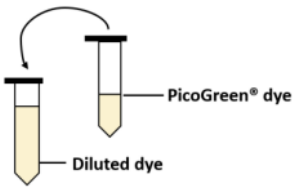


7. Add 500 µL of 2 x TE buffer containing 1% Triton to the samples, mix by gently pipetting and subsequently incubate for 30 minutes at 37°C.

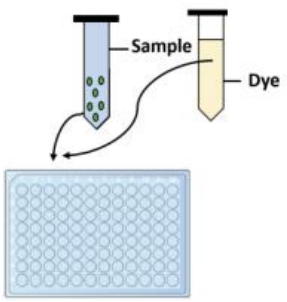


8. Store samples at -20°C for a minimum of 30 minutes, or until ready to quantify DNA.

9. Dilute PicoGreen® dye at a concentration of 1:200 in 2 x TE buffer and wrap the dye solution in aluminium foil to protect the dye from light. Make PicoGreen® dye immediately before use as it is only stable for 1-2 hours.

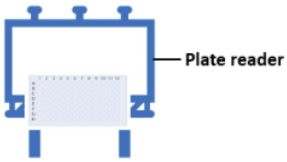


10. Add 100 µL of sample, or standard, in duplicate to a 96-well plate. Add an equal volume of PicoGreen® reagent and mix well by putting the plate on a shaker for 1 minute.



11. Remove from the shaker and incubate for 5 minutes at room temperature (~25°C).

12. Measure the fluorescence at excitation and emission wavelengths of 480 nm and 520 nm respectively. Subtract background fluorescence using the acellular blank.



13. Sample DNA concentration can be calculated by interpolating the sample values in the Lambda DNA standard curve. Total DNA can be calculated by multiplying the DNA concentration by dilution factor (2 for PicoGreen reagent dilution) and sample volume (400 µL pronase + 500 µL 2 x TE buffer with 1% Triton = 900 µL).

For further support, please contact our technical support team at tech@cellgs.com.

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