

QGel™ Protocol

Cell DNA based assays: Example on how to measure the DNA content of cells grown in QGel™ MT 3D Matrix

ABOUT THIS PROTOCOL

This is an example of how to measure proliferation of cells grown in QGel™ MT 3D Matrix using a cell DNA based assays. Further information of these protocols can be also found in published literature (e.g. Bott K. et al, *Biomaterials* 2010). Note that this is only a guideline; volumes and concentrations of the different chemicals and incubation times require to be optimized depending on specific experimental condition.

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Suggested chemicals/solutions/kits:

For gel digestion/cell lysis:

- Proteinase K (Invitrogen™) solution, e.g. 0.5 mg/mL, diluted in phosphate buffered EDTA (5mM EDTA in PBS, pH 7.1).

For DNA measurements:

- CyQUANT™ Cell Proliferation Assay (Invitrogen™)
- OR
- Quant-iT™ PicoGreen® dsDNA Reagent and Kits (Invitrogen™)

Brief procedure description:

1. Cells are encapsulated and grown within QGel™ MT 3D Matrix discs (produced following QGel™ instructions how to make gel discs).
2. After cell culture at specific time points, gel discs harvested for cell DNA measurements are washed in PBS for ca. 20min, and then each gel disc alone is placed in a 1.5 or 2mL plastic tube (e.g. Eppendorf) and stored at -80°C.
3. After thawing, the gel discs are digested and cells lysed by addition of a proteinase K solution to the gel in the eppendorf tube. This happens normally overnight at ca. 60°C, but it could take longer. After this digestion step, the solution should be clear and particle-free.
4. Once the gels are digested and the cells are lysed, the DNA content of the different digestion solutions (prepared in step 1. to 3.) can be subsequently measured using DNA measurements kits (e.g. *Quant-iT™ PicoGreen® dsDNA Reagent and Kits* or *CyQUANT™ Cell Proliferation Assay* – see note n°5 below, both from Invitrogen) following instructions provided by the kit manufacturer.

Important notes/suggestions:

1. We recommend keeping all the gel discs frozen in the different tubes until the collection of the samples from the different time points is completed. DNA measurements can be then performed for all samples from the

different time points and conditions in one single assay section.

2. Normally, two to four gel disc samples with cells per time point per condition are required to ensure consistency of the measurements.

3. Two to four gels *without* cells should also be cultured similarly to those with cells (e.g. just for one time point and one condition) and used as background (e.g. as blank) for the DNA measurement and calculation.

4. Cell proliferation can be represented as the ratio of DNA content per gel at different time points and the DNA content measured in gels frozen immediately (or one day) after cell encapsulation in the discs.

5. If you are using CyQUANT™ GR kit, an additional step for RNA digestion is required before addition of the CyQUANT™ GR dye (see more detailed information in the CyQUANT™ *Cell Proliferation Assay* instructions, chapter of *Experimental Protocol for Cell Number Determination*, and protocol in Bott K. et al, *Biomaterials* 2010). If you are using the most recent version of this assay, called CyQUANT™ NE kit, RNA digestion step is not required anymore.