

QGel™ Protocol

Staining of Nuclei and F-actin Filaments of Cells in QGel™ MT 3D Matrix

ABOUT THIS PROTOCOL

This is an example of staining protocol that can be also found in published literature (e.g. Raeber, Biophysical Journal 2005). Note that the protocol below is only a guideline. Volumes and concentrations of the different chemicals and incubation times require to be optimized for your specific experimental conditions.

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Prepare the following solutions:

- 0.1M Glycine solution in PBS
- Fixation solution/permeabilization: 3-5% Paraformaldehyde (handling precautions are required as it is very toxic) + 0.2% Triton-X100 in PBS at 37°C.
Note: prepare the triton solution separately and add it to the paraformaldehyde solution only at time of fixation.
- 1% w/v BSA solution in PBS
- F-actin filament staining solution: approx. 0.8U/mL (Rhodamine or ALEXA conjugated Phalloidin) in 1% BSA solution in PBS.
Note: You must also follow instructions provided by the manufacturer of the different stains. Rhodamine or ALEXA conjugated Phalloidin are normally stored at higher concentrations and are diluted to working concentrations immediately before use. Concentration may be varied depending on experimental conditions. (caution: phalloidin powder require particular handling precautions as it is very toxic)
- Nucleus staining solution: For example, prepare a 2.5ug/mL solution for DAPI, in PBS
Note: You must also follow instructions provided by the manufacturer of the different stains. Stains are normally stored at higher concentrations and are diluted to working concentrations immediately before use. Other nucleus stains, such as PI, DRAQ5, etc. can also be used instead of DAPI.

A. Fixation and permeabilization:

1. Transfer desired samples in a new well plate (under cell culture hood) and wash them once with PBS at room temperature (RT) for 10 min
2. Add the fixation/permeabilization solution to each gel sample
3. Incubate at RT for 30-50 min (on shaker is preferable but not necessary)
4. 1x wash with 0.1M Glycine at RT for 15-30 min
5. 2x washes with PBS at RT for 5-10 min

Important note: For efficient fixation always make sure that gels with the encapsulated cells are submerged in the different solutions and **not** floating on the surface.

B. Actin filament staining:

(e.g. with Rhodamine-conjugated Phalloidin).

1. Add the F-actin filament staining solution, and incubate at RT for 1-2h (on shaker is preferable but not necessary). **Protect from light!**
2. 2x washes with PBS at RT for 5-10 min (on shaker is preferable but not necessary). **Protect from light!**

Important note: For efficient staining always make sure that gels with the encapsulated cells are submerged in the different solutions and **not** floating on the surface.

C. Cell nuclei staining:

(e.g. with DAPI, DRAQ5, PI, etc.)

1. Add the nucleus staining solution and incubated at RT for 40-50 min (on shaker is preferable but not necessary). **Protect from light!**
2. 2x washes PBS at RT for 5-10 min (on shaker is preferable but not necessary). **Protect from light!**
3. Optional: samples can be mounted in conventional mounting media for longer staining conservation.

Important note: For efficient staining always make sure that gels with the encapsulated cells are submerged in the different solutions and **not** floating on the surface.