ab109204

CyGEL™ Protocol

Instructions for Use

This product is for research use only and is not intended for in vitro diagnostic use.

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1. Introduction

CyGEL™ is a novel thermoreversible hydrogel which is liquid when cold and a gel when warmed.

CyGEL™ is compatible with live cells and can be used to immobilize non-adherent cells by simple warming, and conversely, allowing their recovery by simple cooling.

CyGEL has many applications in imaging and cell-based screening where immobilization is necessary for analysis, while still allowing subsequent release for ongoing experimentation.

Key features of CyGEL™ include:

- Convenient temporal immobilization of non-adherent cells
- Optically clear with low autofluorescence
- Compatible with GFP and “in gel” fluorescent probes including DRAQ5™ (ab108410).

CyGEL is recommended for:

- Mounting live de-adherent and non-adherent cells (primary cells or cell line-derived)
- Mounting live *C. elegans*
- Mounting live parasites such as *Trypanosoma* and *Leishmania*
- Mounting live *Drosophila* embryos for imaging
- Mounting fixed zebrafish for imaging
- Immobilization of live zebrafish for micro-surgery or micro-injections.

2. Contents and Reagents required

- CyGEL™: 8 vials x 500 µl
- PBS: 1 vial of 40x PBS solution (provided with CyGEL™)

Other reagents that might be required for the protocols but are not provided with CyGEL™ are:

- Ice pack
- Microscope slides / chambers
- Coverslips
- Cell buffer (PBS)
- Cell / nuclear dyes

3. Storage and Handling

Before handling ab109204 CyGEL™ please read the MSDS supplied with the product.

Store product at 4°C in the dark. Do not freeze.

Undiluted product is stable for at least 3 years if kept under the recommended conditions.

4. General tips for use of CyGEL™

CyGEL™ changes from a solution when chilled to a gel when warmed quickly, aiding rapid sample preparation and immobilization of cells for visualization.

As supplied, CyGEL™ transits from solution to gel at approximately 23°C (nominally room temperature).

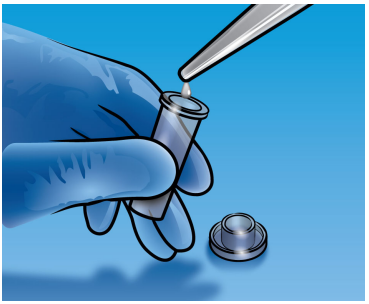
CyGEL™ is formulated to maintain cellular integrity for periods of ≥ 1h for most cell types (and often much longer).

Keep CyGEL™ stocks on ice to facilitate convenient pipetting. If required, ice cold water can be used to “clear” clogged / blocked tips. P1000 displacement tips are recommended for handling CyGEL™.

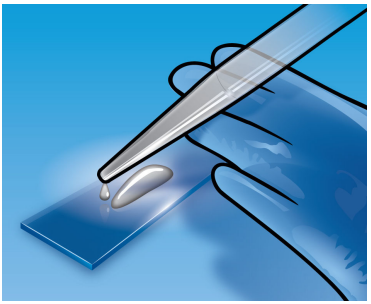
CyGEL™ is very easy to use as demonstrated in five simple steps in the following page.

How to use CyGEL™:

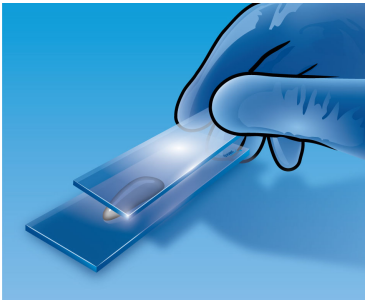
1. Add cells to cooled CyGEL™ and mix gently.



2. Dispense mix onto slide

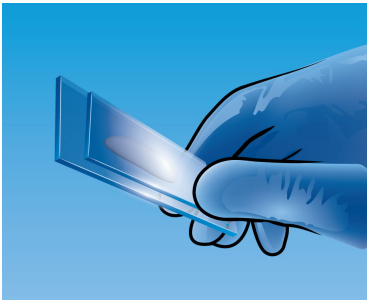


3. Apply coverslip to CyGEL/cells mix



4. Cool gel as this liquefies and spreads the mix

5. Allow slide to warm and re-gel



5. Preparation of CyGEL™ for use

CyGEL™ needs to be primed for use before the addition of cells or tissue to analyze.

1. Select a vial of CyGEL™.
2. Cool the vial on ice for 1 – 2 minutes.
3. Using a sterile pipette tip, add 12.8 µl of the supplied 40xPBS to the CyGEL™ vial. Mix thoroughly, taking care to avoid bubble formation.

The PBS-primed CyGEL™ is now at the correct isotonic strength for the addition of viable cells.

The PBS-primed CyGEL™ will now transit from solution to gel at approximately 20 – 21°C.

6. General Protocols

Protocol I: CyGEL™ mounting of cells onto a standard microscope slide for confocal microscopy / cell imaging.

This general protocol is a guideline, and we recommend adapting it to each user's best protocol.

CyGEL™ is used as mounting media prior analysis of the cells/ embryo.

Preferably, cells should be treated (if required for the experiment) prior to addition of CyGEL™. CyGEL™ can accept certain additives (such as fluorescent dyes) and addition of 1:100 – 1:20 should not affect the performance of CyGEL™. Each new additive should however be tested for impact of gel formation / stability. The overall cell / bead / dye suspension volume should not dilute CyGEL™ by more than 10%; otherwise, the integrity of the hydrogel will be compromised.

1. Cool the PBS-primed CyGEL™ (prepared as described in Section 5) on ice.
2. Prepare cells / embryo according to experiment.
3. Wash cells on cold buffer (e.g. PBS) by centrifugation. Resuspend the cell pellet in a **maximum** of 50 µl cold buffer (typically 1 – 5 x 10^5 cells depending on the application).

4. Pipette the cell suspension into the vial containing PBS-primed CyGEL™ and mix thoroughly but taking care to avoid bubble formation.
5. Transfer 250 µl of the CyGEL™/ cell suspension into a cold P1000 pipette tip. Quickly dispense onto a clean microscope slide by streaking along the surface for a length approaching that of the surface for a length approaching that of the coverslip to be applied.

   NOTE: each PBS-primed CyGEL™ vial contains enough amount to prepare 2 microscope slide preparations.
6. Place the microscope slide onto an ice pack to allow the CyGEL™ to liquefy. The sample will then spread out under the coverslip.
7. Remove the slide from the ice pack. The CyGEL™ will now re-set as it reaches room temperature.

   The sample is now ready for visualization.

Protocol II: CyGEL™ mounting of cells in a chamber coverslip for confocal microscopy / cell imaging.

This general protocol is a guideline, and we recommend adapting it to each user's best protocol.

CyGEL™ is used as mounting media prior analysis of the cells/ embryo.

Preferably, cells should be treated (if required for the experiment) prior to addition of CyGEL™. CyGEL™ can accept certain additives (such as fluorescent dyes) and addition of 1:100 – 1:20 should not affect the performance of CyGEL™. Each new additive should however be tested for impact of gel formation / stability. The overall cell / bead / dye suspension volume should not dilute CyGEL™ by more than 10%; otherwise, the integrity of the hydrogel will be compromised.

1. Cool the PBS-primed CyGEL™ (prepared as described in Section 5) on ice.
2. Prepare cells / embryo according to experiment.
3. Wash cells on cold buffer (e.g. PBS) by centrifugation. Resuspend the cell pellet in cold buffer at a suggested concentration of 2 x 10^6 cells/ml.
4. Pipette 25 µl of the cell suspension into a clean chamber of a microscope 8-chamber coverslip.

5. Transfer 250 µl of the PBS-primed CyGEL™ with a cold P1000 pipette tip and directly overlay the cells in the chamber.
6. Warm the chamber above room temperature (e.g. on a thermally-controlled stage). The CyGEL™ layer will set thereby immobilizing cells and cell clusters for visualization.

Protocol III: CyGEL™ as delivery medium for a cell-permeant dye [DRAQ5™ (ab108410)] in fluorescent imaging of adherent cells.

Preferably, cells should be treated (if required for the experiment) prior to addition of CyGEL™. When that is not possible due to the nature of the experiment, CyGEL™ can accept certain additives such as DRAQ5™.

DRAQ5™ is a cell permeable far-red fluorescent DNA dye that can be used in live cells in combination with common labels such as GFP.

DRAQ5™ should be added to CyGEL™ when is chilled (in solution). Additions of 1:100 – 1:20 should not affect the performance of CyGEL™. Each new additive should however be tested for impact of gel formation / stability. The overall cell / bead / dye suspension volume should not dilute CyGEL™ by more than 10%; otherwise, the integrity of the hydrogel will be compromised.

1. Cool the PBS-primed CyGEL™ (prepared as described in Section 5) on ice.
2. Pipette 2 µl DRAQ5™ (5mM stock solution) and dispense into the PBS-primed CyGEL™ and mix thoroughly. DRAQ5™ is now at a concentration of 20 µM, sufficient for stoichiometric chromatin binding.

3. Prepare cells for mounting in CyGEL™ – DRAQ5™: wash cells on cold buffer (e.g. PBS) by centrifugation. Resuspend the cell pellet in a **maximum** of 50 µl cold buffer (typically 1 – 5 x 10^5 cells depending on the application).
4. Pipette the cell suspension into the vial containing PBS-primed CyGEL™ – DRAQ5™. Mix thoroughly.
8. Transfer 250 µl of the CyGEL™ – DRAQ5™ / cell suspension into a cold P1000 pipette tip. Quickly dispense onto a clean microscope slide by streaking along the surface for a length approaching that of the coverslip to be applied.
   NOTE: each PBS-primed CyGEL™ vial contains enough amount to prepare 2 microscope slide preparations.
9. Place the microscope slide onto an ice pack to allow the CyGEL™ to liquefy. The sample will then spread out under the coverslip.
10. Remove the slide from the ice pack. The CyGEL™ will now re-set as it reaches room temperature.

The sample is now ready for visualization.

DRAQ5™ nuclear binding should be completely equilibrated after 60 – 80 minutes. However, imaging of nuclei will be possible after 20 – 30 minutes.

The individual nuclear fluorescence intensity with DRAQ5™ for each cell measured will reflect the cell cycle age distribution across the population.

Alternative Protocol:

DRAQ5™ can also be added to the cells PRIOR to the addition of CyGEL™.

In that case, follow staining procedure for DRAQ5™ as described in the Protocol and proceed to mount cells in CyGEL™ as described in Protocol I / Protocol II.

DRAQ5™ nuclear fluorescence should be equilibrated after 30 minutes.

Protocol IV: CyGEL™ as delivery medium for a cell-impermeant dye [Propidium Iodide (ab14083)] in time-lapsed fluorescent imaging of membrane – compromised cells.

Preferably, cells should be treated (if required for the experiment) prior to addition of CyGEL™. When that is not possible due to the nature of the experiment, CyGEL™ can accept certain additives such as Propidium Iodide (PI).

Propidium iodide (PI) is a cell impermeable fluorescent DNA dye that is commonly used for nuclei staining on fixed / permeabilized cells or cells with a compromised cellular membrane.

Propidium Iodide (PI) should be added to CyGEL™ when is chilled (in solution). Additions of 1:100 – 1:20 should not affect the performance of CyGEL™. Each new additive should however be tested for impact of gel formation / stability. The overall cell / bead / dye suspension volume should not dilute CyGEL™ by more than 10%; otherwise, the integrity of the hydrogel will be compromised.

1. Cool the PBS-primed CyGEL™ (prepared as described in Section 5) on ice.
2. Pipette 2.5 µl PI (1mg/ml stock solution) and dispense into the PBS-primed CyGEL™ and mix thoroughly. PI is now at a concentration of 5 µg/ml, sufficient for chromatin binding.
3. Prepare cells for mounting in CyGEL™ – PI: wash cells on cold buffer (e.g. PBS) by centrifugation. Resuspend the cell pellet in

a **maximum** of 50 µl cold buffer (typically 1 – 5 x 10^5 cells depending on the application).

4. Pipette the cell suspension into the vial containing PBS-primed CyGEL™ – PI. Mix thoroughly.
5. Transfer 250 µl of the CyGEL™ – PI / cell suspension into a cold P1000 pipette tip. Quickly dispense onto a clean microscope slide by streaking along the surface for a length approaching that of the coverslip to be applied.
   NOTE: each PBS-primed CyGEL™ vial contains enough amount to prepare 2 microscope slide preparations.
6. Place the microscope slide onto an ice pack to allow the CyGEL™ to liquefy. The sample will then spread out under the coverslip.
7. Remove the slide from the ice pack. The CyGEL™ will now re-set as it reaches room temperature.

The sample is now ready for visualization.

Membrane-compromised (i.e., dying / apoptotic) cells will no longer be able to exclude PI and will appear fluorescent under excitation with the appropriate wavelength.

7. FAQs

1. **Which type of cells can I use with CyGEL™?**

   CyGEL™ formulation is compatible with live cells and organisms and can therefore be used with most common biological models that require temporal immobilization for imaging analysis.

   CyGEL can be used on:

   - Live de-adherent and non-adherent cells
   - Cell spheroids
   - *C. elegans*
   - *Drosophila* embryos
   - Parasites such as *Trypanosoma* and *Leishmania*
   - Zebrafish (*Danio rerio*)
   - Non-biological samples such as beads or microspheres.

2. **Can I only use non-adherent cells?**

   No. CyGEL™ can be used on adherent cells previously trypsinized and resuspended in culture media or PBS.

3. **What is the advantage of using CyGEL™ over other immobilization matrices such as LMP (Low Melting Point) agarose or methylcellulose?**

   LMP agarose must be heated above 41°C to remain liquid to allow access to cells, and that temperature is outside the physiological range of most higher organisms. Methylcellulose is opaque and recovery of organisms is difficult.

   CyGEL™ is liquid when cooled and quickly becomes a gel above 21°C. By cooling the gel again it will reliquefy allowing

easy recovery of viable organisms. Moreover, CyGEL™ is optically inert and does not interfere with fluorescence.

4. **Can I use my cells / embryo afterwards?**

   Yes. CyGEL™ (and the accompanying vial of 40xPBS) is provided sterile so cells can used for further experiments. Simply cool the slide/chamber containing CyGEL™ which will cause the gel to liquefy. For recovery of cells, add some cold PBS to dilute the gel (thus preventing it to become a gel again) and wash cells by pelleting them by centrifugation and resuspend in cold PBS/ medium a couple of times; cells are then recovered sterile for further experimentation. Larger objects such as embryos or nematodes can be individually picked out of the liquefied gel and gently washed in the appropriate medium.

5. **Why do you recommend the treatment before the mounting into CyGEL™?**

   CyGEL™, as other gel matrices, will act as reservoir for drugs and other small molecules in the medium and modify its delivery kinetics to the target. If the treatment (such as a pharmaceutical drug or a dye) is added after the cells have been embedded into the CyGEL™, it will take longer for the treatment to reach the target than when cells are on cell culture medium.

   If a delayed delivery of the treatment is desirable, then we recommend adding the treatment after cells are mounted into CyGEL.

6. **Which fluorescent dyes can be used with CyGEL™?**

   CyGEL™ is compatible with most typical fluorochromes. CyGEL™ is optically inert and does not interfere with

fluorescence. Its refractive index is very similar to that of water (1.365 at 37°C as supplied vs 1.33 of water), and changes very little within the operating strength and temperature range.

CyGEL™ allows clear imaging of GFP/ YFP/ RFP – tagged proteins expressed in the cells / embryo of interest. CYGEL™ is also compatible with fluorescent nuclear dyes such as DRAQ5™ (ab108410) and PI (ab14083) and other intracellular markers such as mitochondrial and lysosomal markers.

7. **Can I only use CyGEL™ on live cells?**

   No. The main advantage of CyGEL™ is that allows reversible immobilization of cells/ embryo. This does not preclude however CyGEL™ being used as mountant media for fixed cells stained with fluorochromes linked-antibodies (such DyLight-conjugated antibodies).

8. **In which type of support can I use CyGEL™?**

   CyGEL™ can be used on microscope chambers and glass microscope slides. It can also be used in sterile small petri dish (up to 30 nm diameter) and multi-well plates up to 96wp-size (we do not recommend the use of 384wp as the gel will dry out too quickly and the meniscus formed may distort the image acquisition).

   Bigger objects that might be squashed by a direct contact with coverslip can be placed inside a silicone grease-smeared O-ring, which can then be placed onto a glass microscope slide; the O-ring will act like a mini-well and protect the sample from the direct contact with the coverslip.

9. **What is the difference between CyGEL™ (ab109204) and CyGEL Sustain™ (ab109205)?**

   CyGEL™ is a PBS-based version for general use. CyGEL Sustain™ is a culture medium-ready version designed to enable longer-term imaging or for cells with a high requirement for nutrients or growth factors.

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp