Magnelle® Cell Labeling Kit Protocols

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Magnelle Cell Labeling Kit Contents (BBS-MAGKIT20)

Product	Quantity	Concentration
Magnelle® cell labeling agent	5 vials of 2 mL	4 x 10^9 Magnelles per mL
Anti-Magnelle® antibody	10 µL	1000X
Iron standard	2 vials of 150 µL	1 mM
Iron probe reagent	2 vials of 2 mL	1X
O-ring screw cap tubes	10 tubes	n/a

Storage Conditions and Stability

Store the entire Magnelle Cell Labeling Kit in a -80°C freezer. Note the expiration date on kit components.

Materials required but not included in the Magnelle Cell Labeling Kit:

Cell labeling:

- Antibiotic-free media
- Low oxygen tissue culture incubator (5% O_2, 5% CO_2, 37°C)
- 37°C water bath
- Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS)
- Gentamicin stock solution (50 mg / mL)
- Dissociation solution containing trypsin
- 15 mL conical tubes
- 70% ethanol or isopropanol

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Immunocytochemistry:

- Paraformaldehyde (PFA)
- Triton-X100
- Bovine serum albumin (BSA)
- Anti-rabbit secondary antibody
- Phalloidin stain
- Mounting medium containing DAPI stain

Iron quantification assay:

- Disposable 96-well microplates
- Heat block with block adaptor for microcentrifuge tubes
- Deionized water (ultrapure; purified using a system such as Millipore Milli-Q)
- 1.5 M Hydrochloric acid (molecular biology grade)
- Single channel pipettes and tips
- 1.5 mL microcentrifuge tubes
- Multichannel pipette and reagent reservoir (recommended)
- Microcentrifuge (14-16k x *g*)
- Microplate reader
- PBS
- Vortexer

Introduction

The Magnelle Cell Labeling Kit utilizes the Magnelle reagent to label eukaryotic cells grown in culture. **Magnelles are modified, non-pathogenic bacteria** that deliver a magnetic particle payload and that are then digested. Thus, Magnelles ultimately magnetize target cells and enable *in vivo* cell tracking with standard magnetic resonance imaging (MRI) instruments. Magnelles possess a high relaxivity and enhance T2, T2* MRI contrast within labeled cells.

Applications

The Magnelle labeling protocol has been validated using mesenchymal stem cells (MSCs), induced pluripotent stem cells, neural progenitor cells, cardiomyocytes and cancer cell lines. This protocol has not been tested for cells in suspension. Optimization of labeling may be required for your cells based on initial results. Magnelle labeling efficiency and the iron loading can be assessed by immunocytochemistry (ICC) using the anti-Magnelle antibody and the iron quantification assay, respectively. Protocols are detailed below.

Protocol for Labeling Adherent Cells

Notes:

- **Labeled cells can be cryopreserved after harvesting.**
- **Please read the entire protocol before starting.**

Protocol Timeline:

Day -1	Day 0	Day 1	Day 2+
Passage cells	Label cells with Magnelle reagent for 16 hours	• Wash labeled cells • Incubate cells with gentamicin for at least 4 hours • Harvest cells • Fix cells for ICC	Downstream processes

Preparation

As a starting condition, labeling when cells are 60-70% confluent is recommended. Note that the following procedures have been optimized using adherent human MSC cultures in a 6-well tissue culture plate. This protocol may need to be adjusted depending on the specifics of cell type and objective.

1. 1 day prior to labeling, passage cells in antibiotic-free medium into 6-well plates. Plate an extra well for counting cells in order to calculate the number of Magnelles required.
2. For ICC, grow cells on coverslips or chamber slides to confirm uniform cell labeling across the cell population. See example of ICC results on page 5. Note that the iron quantification assay is used to assess labeling efficiency (see further below).

Resuscitation of Frozen Magnelle Reagent

3. Set a water bath to 37°C.
4. Whilst maintaining a constant hold of the frozen vial(s), place into a 37°C water bath to no more than half the depth of the vial.

   Note:

   - Do not submerge vials in the water bath in order to prevent extravasation of water into the vial(s).
   - We recommend that you work with no more than two vials at a time to be able to closely monitor thawing.
5. Swirl the vial(s) slowly and constantly while partially submerged.
6. Remove the vial(s) when approximately 80% of thawing has occurred. A vial of Magnelles takes approximately 3 minutes to thaw when placed at 37°C.

   Note: The remaining thawing will occur in the next minute.
7. Disinfect the outside of the vial by either spraying or wiping down with 70% ethanol (or equivalent) prior to placing the vial(s) in a Class II biosafety cabinet.

Labeling

We recommend starting exploratory experiments at a Magnelle labeling ratio (MLR) of 2000 Magnelles per cell and adjusting the MLR according to the sensitivity of the cells. Note that the MLR is the ratio of Magnelles to eukaryotic cells added at the beginning of the labeling procedure and does not describe the actual number of Magnelles entering per cell. The minimally effective MLR is the lowest ratio required to adequately label the eukaryotic cells for detection with your T2 MRI instrument.

8. Obtain a cell count from at least one well of cells.
9. Using your cell count, calculate the volume of Magnelle reagent required. Magnelles are provided at a concentration of 4 x 10^9 Magnelles/mL.

   Example calculation

$$\frac{2000\ MLR\ \times\ cell\ count\ per\ well\ \times\ \#\ of\ wells}{4 \times 10^9 Magnelles/mL} = required\ volume\ of\ Magnelles$$

10. Invert the thawed Magnelle reagent vial(s) several times to partially resuspend Magnelles.
11. Open the vial and transfer part of the suspension (~1 mL) to a 15 mL conical tube.
12. Mix the remaining content of the vial by pipetting up and down 2-3 times with a 1 ml single channel pipette, and transfer to the same 15 mL conical tube as in step 11.

    Note: Contents of several Magnelle reagent vials can be mixed if needed.
13. Centrifuge Magnelles at 3000 x *g* for 15 minutes. Carefully remove supernatant above the Magnelle pellet.
14. Resuspend the pellet in antibiotic-free cell culture medium such that 1.5 mL of medium can be applied per well, at the desired MLR.
15. Aspirate existing medium in your tissue culture plate and discard appropriately. Add 1.5 mL of antibiotic-free medium containing Magnelles to each well of cells to be labeled.
16. Incubate cells and Magnelles for 16 hours in a low oxygen incubator (5% O_2, 5% CO_2, 37°C).

Washing

17. Following incubation with Magnelles, wash cells 5 x with 2 mL Ca^{2+}- and Mg^{2+}-free PBS.
18. Following the wash steps, add 2 mL of cell culture medium containing 50 µg/mL gentamicin and incubate cells for a minimum of 4 hours prior to harvesting.

Harvesting

19. After incubation, remove the gentamicin-containing culture medium.
20. For ICC, do not add dissociation solution. Proceed to the ICC protocol below. Add dissociation solution to wells from which cells will be harvested for the Iron Quantification Assay. When the cells have detached, stop the dissociation with cell culture medium and transfer the cell suspension into a conical tube. Wash the tissue culture dish with additional cell culture medium and add it to the conical tube.
21. Spin down cells as appropriate for your cell type.

22. For iron loading quantification, spin down the cells and resuspend in PBS. Obtain a cell count and proceed to the iron quantification assay protocol.

Immunocytochemistry Example Protocol

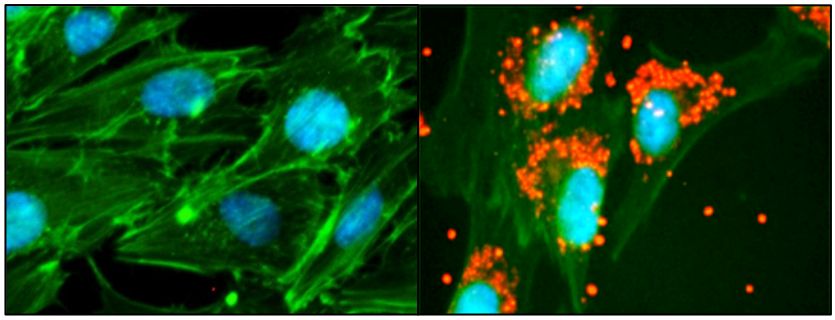


Figure 1: ICC images of hMSCs labeled with Magnelles in red (right) and control unlabeled cells (left) at 40X magnification.

Note that the following is an example protocol. ICC protocols routine to your laboratory may be followed.

1. Aspirate media and wash coverslips 2X with PBS to remove residual serum.
2. Fix labeled cells in 4% PFA for 10 minutes. Wash 3X with PBS.
3. Permeabilize cells using 0.1% Triton X-100 diluted in 1% BSA for 5 minutes at room temperature. Wash 3X with PBS.
4. Block coverslips in 1% BSA for 30 minutes - 1 hour at room temperature.
5. Stain cells with anti-Magnelle antibody diluted 1:1000 in 1% BSA for 1 hour at room temperature or 20 minutes at 37°C. Wash 3X with PBS.

   Note: Overnight incubation is not recommended.
6. Incubate cells with an anti-rabbit secondary antibody and Phalloidin for 1 hour at room temperature or 20 minutes at 37°C. Wash 3X with PBS.
7. Mount cells in mounting medium containing DAPI and cure or seal as appropriate. Cells are now ready for imaging.

Iron Quantification Assay

The iron quantification assay is a simple methodology to estimate the total iron concentration in Magnelle-labeled cells.

Protocol for Iron Quantification Assay

Sample preparation

1. Harvest 5 x 10^5 Magnelle-labeled cells in the provided 2 mL screw cap tubes. It is critical to use screw cap tubes containing O-rings to prevent evaporative sample loss during the subsequent heating step. Pellet the cells as appropriate, remove the supernatant and resuspend in 150 µL PBS.

Note: We recommended running samples in triplicates, or at least in duplicates to test labeling efficiency results.

2. Add 100 µL 1.5 M hydrochloric acid to each sample. Vortex samples to mix.
3. Pulse-spin tubes to force all sample to the bottom of the tubes.
4. Place the samples in a heat block at 75°C and heat for 2 hours.

   Note: It is recommended to fill the heat block with sand or fine glass beads for optimal heat transfer to the tubes.

5. Vortex the sample tubes every 30 minutes to improve sample digestion. Pulse-spin samples for after each vortex.
6. Allow the samples to cool at room temperature for 30 minutes. While waiting proceed to step 6.

Iron Quantification Assay Procedure

7. Warm the iron probe reagent and the iron standard to room temperature before use (~30 minutes). Vortex and briefly centrifuge vials prior to use.
8. Preparation of solutions to generate an iron standard curve:
   - This assay kit is optimized to detect iron concentrations within a range of 2 to 20 nmol in a sample volume of 200 µL.
   - Prepare a 0.1 mM iron standard solution by adding 1350 µL PBS to the supplied 1 mM iron standard solution. Vortex the solution.
   - The iron concentrations required for the standard curve are listed in the table below (Column 1).
   - Add the appropriate volume of the 0.1 mM diluted iron standard solution (Column 2) to a 96-well microplate. Bring the volume of each well up to 200 µL using PBS (Column 3). Each standard should be prepared in duplicate.

   Note: For samples with lower than 5 x 10^5 cells or when a low degree of labeling is expected, a 0.5 nmol and 1 nmol iron standard should also be prepared.

Column 1	Column 2	Column 3
Iron concentration (nmol)	Volume of 0.1 mM stock (µL)	Volume of PBS (µL)
0 (Blank)	0 (Blank)	200
2	20	180
4	40	160
6	60	140
8	80	120
12	120	80
16	160	40
20	200	0

9. Place samples in the 2 mL screw cap tube into a microcentrifuge and spin at maximal speed (14-16k x *g*) for 5 minutes to pellet insoluble cell debris.

10. Transfer 220 µL of sample supernatant into a 1.5 mL microcentrifuge tube, taking care not to disturb the pellet. Spin samples at maximum speed for 5 minutes to remove cell debris that may have carried over.
11. Carefully transfer 200 µL of sample supernatant into a 96-well microplate, making sure not to transfer any cell debris.

    Note: An example calculation is provided at the end of this document illustrating how to account for the portion of the processed sample not used when calculating the iron concentration per cell.
12. Use a single-channel pipette, or preferably a multichannel pipette, to add 60 µL of iron probe reagent to all wells containing the standards and the samples to be tested. Mix samples thoroughly by pipetting up and down.
13. Incubate the microplate for one hour at room temperature in the dark.
14. Measure the absorbance at 570 nm using a microplate reader.

Calculations

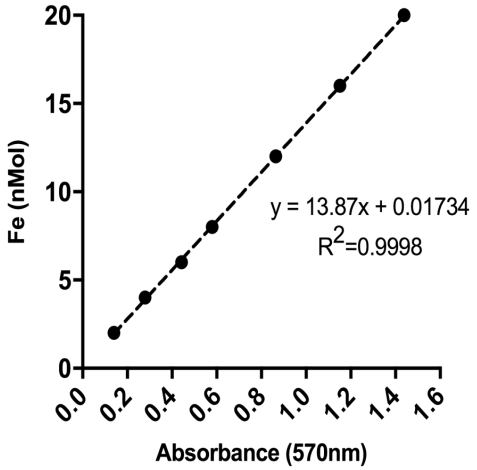
15. Calculate the average absorbance reading for the blanks and standards. Subtract the average absorbance reading of the blank from that of each standard and sample.
16. Generate a standard curve (see example below) by plotting the average blank-corrected absorbance for each standard against its known iron concentration in nmol.

    Note: Coefficient of determination (R^2) values of at least ≥ 0.98 are routinely obtained.
17. Use the equation generated from the standard curve to calculate total iron (nmol) in each unknown sample.
18. Use the formula below to convert nmol of iron in each unknown sample to picogram iron per cell (pg Fe/cell).

    Note: 200µl of 250µl is assayed, therefore 80% of the total cells are assayed.

$$\frac{Fe\ (pg)}{cell} = nmol\ Fe \times 0.055845 \frac{\mu g\ Fe}{nmol\ Fe} \times \frac{10^6 pg}{\mu g} \times \frac{1}{number\ of\ cells\ in\ assayed\ volume *}$$

Iron standard curve example



Troubleshooting

Significant decrease in cell viability	Lower MLR.
	Attempt labeling again using freshly plated, healthy cells.
	Increase cell density.
Magnelle labeling of cells is low	Increase MLR.
	Simulate the optimal environment for your cells during the labeling process (preferred temperature, media, confluency, etc)
	Attempt labeling again using freshly plated, healthy cells.
	Confirm that Magnelles are stored correctly and are not used beyond their shelf life date.

Frequently Asked Questions

1) Do I need to have a low oxygen incubator in order to label cells?

For best results, a 5% low oxygen incubator is recommended as labeling under low oxygen conditions has resulted in the most consistent, highest efficiency labeling. Use of regular tissue culture incubator has not been tested and is down to the investigator's judgement.

2) Do I have to label my cells in 6-well plates? Can I label in other plates or flasks?

Standard tissue culture vessels may be used to label cells with Magnelles. Be sure to adapt the volumes in the labeling protocol accordingly if using tissue culture ware other than a 6-well plate. As a general rule, try to minimize the surface area in order to maximize Magnelles labeling efficiency.

3) What Magnelle dose (MLR) should I use for labeling?

The Magnelles dose you use is dependent on your cell type and its endocytic capacity. Doses ranging from 100 to 8000 MLR have been tested. The optimal Magnelle dose may need to be experimentally determined. A general rule of thumb is to optimize MLR by keeping MLR as low as possible and minimally altering cell characteristics while still producing an MRI signal.

Example of MLRs and cell line:

Cell type	MLR
Mesenchymal stem cells	2000
Neural progenitor cells	2000
Cardiomyocytes	5000
Cancer cells	2000-5000

4) Can I label suspension cells?

Magnelle labeling has been tested and validated with adherent cell types, but Magnelles may be used to label suspension cells depending on their endocytic capacity. Please contact technical support if you are interested in trying to label a suspension cell type.

5) Can I freeze down and thaw Magnelle-labeled cells?

Magnelle-labeled cells can be frozen down, thawed and used for downstream applications. However, it is not recommended to subculture Magnelle-labeled cells as signal may be diluted as cells divide.

6) Does Magnelle labeling affect my cell's functionalities and morphology?

Magnelle labeling has been tested on a wide variety of cells with little to no changes in cell functionality and morphology. If functional or morphology changes are observed, try labeling with a lower MLR. While the ICC and Magnelle iron assay are included in the kit, we also recommend performing functional assays specific your cell type to confirm Magnelle labeling does not have any effect on your cells.

7) Can we use Magnelles to label cells that grow in clusters or spheres?

Experiments with induced pluripotent stem cells, which grow as spherical clusters, showed differential labeling efficiency based on depth in the cluster. This observation should be taken into account in experiments to visualize cells *in vivo* by MRI.

8) What sterility precautions should I take while working with Magnelles in my biosafety cabinet?

While the Magnelle is a live bacterium, it is readily inactivated by standard cleaning procedures (e.g. wiping with 70% ethanol or isopropanol and UV light). We recommend strict adherence to aseptic technique and disinfection of work areas directly exposed to Magnelles.

For additional technical support, please contact us at info@bellbiosystems.com.

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