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Preparation of live cell samples for fluorescence spectroscopy and computational super-resolution imaging

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Method Article

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Abstract

We describe a protocol for the preparation of live cell samples for fluorescence spectroscopy and computational super-resolution imaging. We detail here how to culture, transfect, and prepare the cells for fluorescence applications.

Introduction

Cell culture is the cultivation of cells derived from an animal or plant source in an artificially controlled environment. It is a major model system in cell and molecular biology [1][2]. Fluorescence microscopy of live cells allows investigation of cellular processes in a non-destructive manner. Fluorescence is highly sensitive, and single-molecule detection is possible [3][4][5].

We detail here a protocol to transfect fluorescent proteins and prepare live cell samples for fluorescence microscopy. While this protocol uses CHO-K1 cells, it can be used for most adherent cell types with suitable modifications as required.

Reagents

1. Dulbecco's Modified Eagle Medium (DMEM/High glucose with L-glutamine, without sodium pyruvate – #SH30022.FS; HyClone, GE Healthcare Life Sciences, Utah, USA).

2. Penicillin-streptomycin (#15070063, Gibco, Thermo Fisher Scientific, Massachusetts, USA).

3. Fetal bovine serum (FBS; #10270106, Gibco, Thermo Fisher Scientific, Massachusetts, USA).

4. 1X phosphate buffered saline (PBS; without Ca^{2+} and Mg^{2+}).

5. 10X Trypsin-EDTA (0.5%, no phenol red; #15400054; Gibco, Thermo Fisher Scientific, Massachusetts, USA). Dilute to 1x working solution. Alternatively, TrypLE (Gibco, Thermo Fisher Scientific, Massachusetts, USA) or a cell scraper can be used.

6. Suitable amounts of highly pure (A_{260}/A_{280} > 1.8) plasmids.

7. Neon Transfection Kit (Thermo Fisher Scientific, Massachusetts, USA).

8. 1X Hank's Balanced Salt Solution (HBSS, with Ca^{2+} and Mg^{2+}; #14025134; Gibco, Thermo Fisher Scientific, Massachusetts, USA). Alternatively, 1X PBS (containing Ca^{2+} and Mg^{2+}) can be used.

9. Imaging DMEM (#21063029; Gibco, Thermo Fisher Scientific, Massachusetts, USA).

Equipment

1. Biosafety cabinet (Labgard ES (Energy Saver) Class II, Type A2 Laminar Flow Biological Safety Cabinet; NuAire, Minnesota, USA).

2. CO_2 incubator to maintain 37°C and 5% CO_2 incubator (Forma Steri-Cycle CO_2 incubator, Thermo Fisher Scientific, Massachusetts, USA).

3. Glass Bottom Dishes suitable for imaging (#P35G-1.5-20-C, MatTek, Massachusetts, USA).

4. Centrifuge (#5810, Eppendorf, Hamburg, Germany).

5. Automated cell counter (TC20, Bio-Rad, Singapore). Alternatively, a hemocytometer can be used.

6. Glass Bottom Dishes suitable for imaging (#P35G-1.5-20-C, MatTek, Massachusetts, USA). An alternative product is Nunc™ Lab-Tek™ II Chambered Coverglass (Thermo Fisher Scientific, Massachusetts, USA).

7. 15 ml polypropylene conical tubes

8. Polypropylene microcentrifuge tubes.

9. Micropipettes of different volume ranges and corresponding sterile tips.

10. Pipette controller and serological pipettes of various volumes.

11. 70% ethanol.

12. Cell imaging microscope with fluorescence imaging facility (EVOS fl, Advanced Microscopy Group (AMG), Washington, USA).

Procedure

1. Wipe the biosafety cabinet thoroughly with 70% ethanol. Also spray and wipe down all items taken inside the cabinet with 70% ethanol.

2. Warm all media and solutions to 37°C in a water bath before use.

3. Culture CHO-K1 (Chinese Hamster Ovary) cells (CCL-61) in DMEM (high glucose with L-glutamine, without sodium pyruvate) supplemented with 1% penicillin-streptomycin and 10% FBS at 37 °C in a 5% (v/v) CO_2 environment. Once the culture is ~90% confluent it can be used for transfection.

4. Remove the spent media from the culture flask and discard.

5. Wash the flask twice with 5 ml PBS (without Ca^{2+} and Mg^{2+}).

6. Add 2 ml Trypsin-EDTA (1X) and incubate the flask at 37°C for 5-15 minutes to detach the cells.

7. Add 5 ml culture media (containing serum) to the flask to inhibit trypsin.

8. Collect the media containing the detached cells in a falcon tube and centrifuge at 200 × g for 3 minutes.

9. Discard the supernatant and resuspend the cell pellet in 5 ml PBS.

10. Count the cells using a cell counter.

11. Aliquot the required number of cells (typically 0.5-1 million) into falcon tubes and centrifuge at 200 × g for 3 minutes.

12. Discard the supernatant and resuspend the cell pellet in R buffer (Neon Transfection Kit).

13. Mix suitable amounts of plasmids with the cells for transfection.

14. Electroporate the cells using Neon Transfection system according to the manufacturer's protocol (electroporation settings: pulse voltage = 1,000 V, pulse width = 30 ms, and pulse no. = 2).

15. After transfection, seed the cells onto glass bottom culture dishes containing DMEM (supplemented with 10% FBS; no antibiotics).

16. Incubate the cells at 37°C and 5% CO_2 for 36-48 hours before experiments.

17. Observe the cells under a fluorescence microscope to check for fluorescence and determine whether the transfection was successful.

18. Remove the spent media and wash 2-3 times with 1X HBSS. Then add DMEM not containing phenol red (Imaging DMEM) to reduce background fluorescence.

Note: If cell starvation is required, do not supplement the Imaging DMEM with FBS. Also, if a 5% CO_2 environment cannot be maintained on the microscope setup, supplement the Imaging DMEM with HEPES.

19. The transfected cells can now be used for experiments.

20. If any ligand stimulation or drug treatment needs to be done, prepare suitable concentration of the drug or ligand in Imaging DMEM and add the solution to the cell culture dish.

Troubleshooting

1. If cells do not get transfected well:

• Increase the plasmid amount used. Larger plasmids require more amounts to get successfully transfected.

• Optimize the electroporation settings. While the manufacturer provides electroporation parameters for a number of cell lines, this might need to be optimized further. Alternatively, a lipid-based transfection reagent (e.g. Lipofectamine 3000, FugeneHD, etc.) can be tried.

• Cells which are of lower passage number will usually get transfected better. We typically avoid using cells with passage numbers > 20.

2. After transfection, the cells will need time (typically at least 24 hours) to recover their normal morphology and characteristics.

• While we usually use the cells 36-48 hours after transfection for experiments, they can be used anytime from 24 to 72 hours after transfection as long as the cells are healthy and show normal morphology.

3. Contamination issues:

• Always handle cells in a biosafety cabinet and practice proper aseptic techniques.

• Ensure the equipment, media and other reagents used are sterile.

• Ensure the antibiotic used is not degraded and of correct concentration (*Note*: good cell culture practice is to avoid using antibiotics in the first place; if proper aseptic techniques are practiced, antibiotics are not required).

4. Wash steps with buffers:

• The buffers used for washing prior to trypsinization should not contain Ca^{2+} and Mg^{2+} as these ions promote cell adhesion.

• Conversely, the buffers used for washing prior to imaging should contain Ca^{2+} and Mg^{2+} to avoid detachment of cells.

5. Trypsinization

• While cells will usually detach quickly upon trypsinization, it can take upto 15 minutes depending on the cell type and the potency of the trypsin used. Avoid old trypsin stocks or stocks that have been subjected to temperature changes multiple times.

• Do not use very high trypsin concentrations or leave the cell culture for too long in trypsin as it will kill the cells. If the cells are not detaching easily, a cell scrapper can be used as an alternative.

• The culture media used for neutralizing the trypsin after cell detachment should contain serum. Serum contains protease inhibitors that inhibit the activity of trypsin.

Time Taken

A freshly passaged cell culture would be confluent in 2-3 days depending on the initial number of cells seeded. The electroporation protocol would take about an hour. Post-transfection recovery of the cells would take atleast 24 hours.

Anticipated Results

Fluorescence can be observed in the cells a few hours after transfection. After the cells have recovered post-transfection and regained their normal morphology, they can be used for experiments.

References

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