BUKTI KORESPONDENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul artikel	:	Affordable and Simple Protocol for Immunofluorescence Staining of Insulin Secreting iGL Cell Line 2D and 3D
Jurnal	:	Journal of Experimental and Clinical Medicine https://dergipark.org.tr/omujecm, 2024, volume 41(3), 590 – 594
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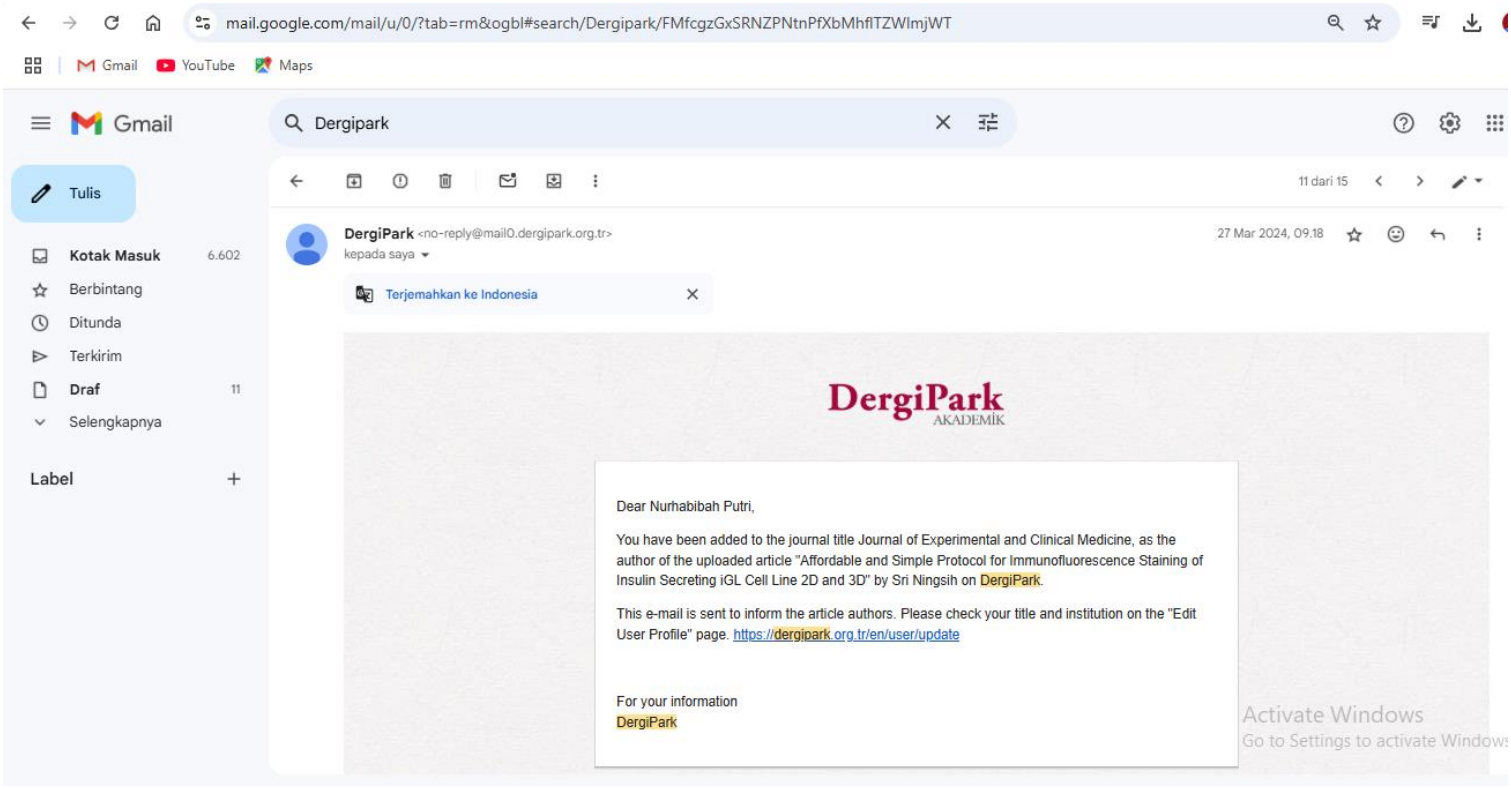
No.	Perihal	Tanggal
1.	Bukti konfirmasi submit artikel dan artikel yang disbumit	27 Maret 2024
2.	Bukti konfirmasi review dan hasil review pertama	29 Maret 2024
3.	Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit	29 Maret 2024 – 4 April 2024
4.	Bukti konfirmasi review dan hasil review kedua	9 Mei 2024
5.	Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit	9 Mei 2024 – 29 Juni 2024
6.	Bukti konfirmasi artikel accepted	8 Juli 2024
7.	Bukti konfirmasi artikel published online	30 Agustus 2024

1. Bukti Konfirmasi Submit Artikel dan Artikel yang Disubmit (27 Maret 2024)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J Exp Clin Med
2024; 41(3): 590-594
doi: 10.52142/omujecm.41.3.24

Affordable and simple protocol for immunofluorescence staining of insulin secreting iGL cell line 2D and 3D

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Received: 27.03.2024 • **Accepted/Published Online:** 08.07.2024 • **Final Version:** 30.09.2024

Abstract

Immunofluorescence staining is a widely used technique to visualize the localization of specific proteins in cells. This protocol provides an affordable and simple method for immunofluorescence staining of the Insulin-GLase (iGL) cell line in both 2D and 3D culture conditions. We describe cost-effective alternatives for reagents and materials without compromising the quality of the results, such as the use of Bovine Serum Albumin (BSA) 3% as a blocking buffer instead of normal serum. Immunofluorescence staining on the iGL cell line is a valuable technique for visualizing the distribution and localization of target biomolecules. With careful consideration of cost-effective strategies and an understanding of the unique characteristics of the iGL cell line, it is possible to achieve reliable and informative immunofluorescence results even with budget constraints.

Keywords: cell culture, immunofluorescence, iGL, 2D and 3D culture

1. Introduction

Immunofluorescence staining is a powerful technique to visualize the distribution and localization of specific biomolecules within biological samples, such as cells and tissues. It involves using antibodies linked to fluorophores, which emit fluorescence when excited with specific wavelengths of light. Immunofluorescence can provide detailed information about the localization, expression levels, and distribution of target proteins within a sample (1-3). Insulin-GLase (iGL) is a cell line developed for studying insulin production and regulation. This cell line is derived from INS-1E rat insulinoma cells and MIN6 cells, which are known to display beta-cell characteristics and secrete insulin in response to glucose (4). It is important to understand the characteristics of the iGL cell line to optimize the immunofluorescence staining protocol effectively.

The iGL cell line is widely used for studying the expression and localization of specific biomolecules within the cells. Its unique features and growth patterns make it a valuable model for immunofluorescence studies. The immunofluorescence analysis of the iGL cell line allows researchers to visualize and quantify the expression of specific proteins, including insulin, within the cells (3).

Immunofluorescence was performed in the iGL cell line using various techniques, such as co-transfection with plasmids expressing FLAG-tagged ChREBP and Mlx and

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staining for insulin using specific antibodies (5-6). The immunofluorescence analysis of the iGL cell line revealed robust glucose-stimulated insulin secretion and the expression of ChREBP, a transcription factor involved in regulating insulin production. Furthermore, the iGL cell line displayed a uniform pattern of insulin immunofluorescence that closely resembled normal islets within the pancreas (7). Overall, immunofluorescence analysis of the iGL cell line provided valuable insights into the expression and regulation of insulin (5, 8-10).

This study can provide valuable insights into an affordable and simple protocol for immunofluorescence staining of the iGL cell line. Using immunofluorescence analysis, researchers can confirm the successful differentiation of iGL cells into functional beta cells that produce insulin. Additionally, immunofluorescence analysis can be used to evaluate the response of iGL cells to glucose stimulation. For example, if the iGL cell line exhibits robust glucose-stimulated insulin secretion, this suggests that the cells are responsive to changes in glucose levels and capable of producing insulin in response to physiological demand.

2. Materials and Methods

2.1. iGL cell 2D and 3D culture system iGL cells derived from Cosmo Bio, IGL01C, Japan were thawed and cultured in complete media. The complete media was produced using the following recipe:

1. RPMI1640 (containing L-glutamine, phenol red, and HEPES) as the basal media (Gibco, 11875093,USA) Additives :
2. 5% of FBS (Fetal Bovine Serum) (PAN Biotech, P303306, South America)
3. 1 mM Sodium Pyruvate (Gibco, 11360070, USA)
4. 500 µM monothioglycerol (Sigma-Aldrich, 96275, USA) 5. 200 µg/mL G-418 (Sigma-Aldrich, 108321422, USA)

First, the base media was prepared: RPMI1640 containing L-glutamine, phenol red, and HEPES (Gibco, 11875093,USA). The media was then supplemented with 5% of FBS (Fetal Bovine Serum) (PAN Biotech, P303306, South America), 1 mM pyruvic acid (Gibco, 11360070,USA) 500 µM monothioglycerol (Sigma-Aldrich, 96275, USA), 200 µg/mL G-418 (Sigma-Aldrich, 108321422, USA) into the base media (Cosmo Bio, IGLM, Japan). After that, frozen cells were thawed in vials in a water bath at 37 °C for two minutes. (Cosmo Bio, IGLM, Japan). We added 1 mL of complete media into the vials, then transferred the cell suspension into 15 mL tubes (Corning, 430791, USA) with 9 mL of complete media. Next, the cells were centrifuged at a speed of 300 g for five minutes, and then the supernatant was discarded. Pellets of cells were resuspended with 1 mL of medium and counted with an automatic cell counter (LUNA Automated Cell Counter, Logos Biosystem, 0300119, South Korea). The cell suspension was seeded into a 100-mm dish (Corning, 430167, USA) and incubated at 37 °C in 5% CO_2. It was replaced with fresh medium every three to four days. After reaching 70-90% of confluency, the cells were passaged. (11)

After cell propagation, cells were collected and divided into two culture systems. The first is a 2D or monolayer culture which was conducted in 6-well culture plate (SPL, 13485, South Korea) with 50.000 cells per well confluency. The other is a 3D culture system using the hanging drop method. The cell suspension drops were formed by placing 25 µL cell suspension on the inner part of the bacterial petri dish lid of a 100×20 mm bacterial petri dish (SPL,10101, South Korea). The cell suspension drops were placed in a relatively identical distance at the inner part of the bacterial petri dish lid. Forty drops were prepared for each cell concentration performed in one petri dish. Once all the drops were completed, the lid was flipped quickly and carefully to maintain the drops. The petri

dishes were placed in the incubator at 37 °C with 5% of CO_2 for two days. After the 3D culture system was harvested, we then continued with the 3D iGL cell line immunofluorescence method. (11)

2.2. 2D iGL cell line immunofluorescence method

After cell propagation, the cells were passaged up to the 9th passage. Subsequently, the cells were harvested, and cell staining was initiated. For iGL cell line immunofluorescence

analysis in 2D, the following procedure was followed. After the cells were seeded and harvested in a 24-well cell culture plate (Corning, 3524, China), the iGL cells were fixed with glass coverslips (coverslips are optional). Next, they were rinsed once with warm PBS (Gibco, 18912014, USA) and allowed to sit briefly, and then the PBS solution was carefully discarded using a pipette. After that, the cells were covered to a 2–3 mm depth with a warm fixation solution of 10% Neutral Buffered Formalin (Leica Biosystem, 3800698, USA) and fixed for 15 minutes at room temperature.

After incubating for 15 minutes at room temperature, the fixation solution is carefully discarded using a pipette. The cells are rinsed three times in 1X PBS (Gibco, 18912014, USA) 300 µl per well for five minutes each time. Permeabilization solution 0.3% Triton X-100 is added in PBS (Invitrogen, HFHF10, USA) at 100 µl per well and incubated for five minutes at room temperature. Then, it is washed once with Milli-Q/aquabidest, 300 µl per well, and after that 200 µl per well of blocking buffer, Bovine Serum Albumin (BSA) 3% (Sigma-Aldrich, 1003269866, USA) is added to the chamber sections and incubated for one hour at room temperature. After incubating for one hour, the blocking buffer is discarded and the primary antibody solution is added in the amount of 200 µl per well to the chamber slides and incubated at room temperature or overnight at 4 °C. The recommended concentrations on the datasheet in the antibody dilution buffer are 1: 200 (PBS = 995 µl + connexin 36 = 5 µl), 1: 100 (PBS = 990 µl + connexin 36 = 10 µl), 1: 50 (PBS = 980 µl + connexin 36 = 20 µl), 1: 10 (PBS = 900 µl + connexin 36 = 100 µl). After being incubated overnight at 4 °C, it is washed three times in PBS (Gibco, 18912014, USA) 300 µl per well.

A fluorochrome-conjugated secondary antibody, goat antiRabbit IgG (H+L) Secondary Antibody, FITC (Invitrogen, 656111, USA), is prepared and diluted and added at 200 µl per well. The secondary antibody dilution consisting of (1: 1000; PBS = 999 µl + FITC 1 µl) is then incubated for two hours at room temperature in the dark. Next, it is washed three times with Milli-Q/aquabidest, 300 µl per well. Then, DAPI (Thermo Scientific™, 62248, USA) is added, diluted 200 µl per well, in the dark. The DAPI dilution consisting of (1: 1000; PBS = 999 µl + DAPI 1 µl) is then incubated for 10 minutes at room temperature in the dark, or the coverslips are mounted onto microscope slides using mounting media with a nuclear stain, such as DAPI, to visualize the cell nuclei.

After the staining steps are complete, we visualize and quantify the marker expression and localization within the iGL cell line using fluorescence microscopy (Nikon, Ts2 Ph Fl, Japan) integrated with a camera (Nikon, DS-Fi3, Japan).

2.3. 3D iGL cell line immunofluorescence method

After the cells were harvested (hanging drop method) in a 100×20 mm bacterial petri dish (SPL, 10101, South Korea) the first step in immunofluorescence staining for the 3D iGL cell line is to prepare a pap pen circle onto object glass. Then, the spheroid area is carefully transferred using a micropipette (Appendorf, USA) inside the pap pen and incubated for 30 minutes at room temperature. After that, cells are covered to 2– 3 mm depth with a warm fixation solution of 10% Neutral Buffered Formalin (Leica Biosystem, 3800698, USA) and fixed for one hour at room temperature. After incubating one hour at room temperature, the fixation solution is discarded. The cells are then rinsed three times in 1X PBS (Gibco, 18912014, USA) for five minutes each time. Permeabilization solution 0.3% Triton X-100 in PBS (Invitrogen, HFHF10, USA) is added, 300 µl per object glass (pap pen area) and incubated for five minutes at room temperature. Then, the cells are washed once with Milli-Q/aquabidest, and then 200 µl blocking buffer, Bovine Serum Albumin (BSA) 3% (SigmaAldrich, 1003269866, USA), is added to the chamber sections and incubated for one hour at room temperature.

After incubating for one hour, the blocking buffer is discarded and the primary antibody solution is added in the amount of 200 µl to the chamber slides and incubated at room temperature or overnight at 4 °C. The recommended concentrations on the datasheet in the antibody dilution buffer are 1: 200 (PBS = 995 µl + connexin 36 = 5 µl), 1: 100 (PBS = 990 µl + connexin 36 = 10 µl), 1: 50 (PBS = 980 µl + connexin 36 = 20 µl), 1: 10 (PBS = 900 µl + connexin 36 = 100 µl). After incubating overnight at 4 °C, wash three times in PBS (Gibco, 18912014, USA). A fluorochrome-conjugated secondary antibody, goat anti-Rabbit IgG (H+L) Secondary Antibody, FITC (Invitrogen, 656111, USA), is prepared and diluted and 200 µl is added per object glass (pap pen area).

The secondary antibody dilution consisting of (1: 1000; PBS = 999 µl + FITC 1 µl) is then incubated for two hours at room temperature in the dark. Next the cells are washed three times with Milli-Q/aquabidest. Then, DAPI (Thermo Scientific™, 62248, USA) is added, diluted, 200 µl per object glass (pap pen area). This is done it in the dark. The DAPI dilution consisting of (1: 1000; PBS = 999 µl + DAPI 1 µl) is then incubated for 10 minutes at room temperature in the dark, or the coverslips are mounted onto microscope slides using mounting media with a nuclear stain, such as DAPI, to visualize the cell nuclei. After the staining steps are complete, the marker expression and localization within the iGL cell line are visualized and quantified using fluorescence microscopy (Nikon, Ts2 Ph Fl, Japan) integrated with

a camera (Nikon, DS-Fi3, Japan).

3. Results

3.1. Microscopic observation results of 2D and 3D cultures of iGL cells

The monolayer culture of iGL cells reached more than 70% confluency after two days of incubation (Fig. 1). This type of adherent cells aggregated to each other as per typical characteristics in natural conditions. In the 3D culture system, the aggregated cells formed a compact round-shaped structure called a spheroid (Fig. 2). The spheroid began to form from the first day after cell seeding with average size.

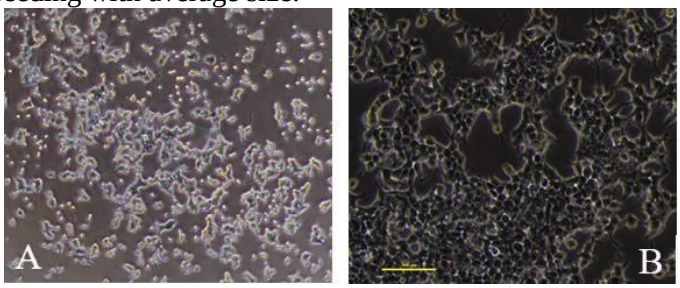

Fig. 1. Monolayer cell culture of iGL cell. Phase contrast microscope, mag. 40x (A) and 100x (B)

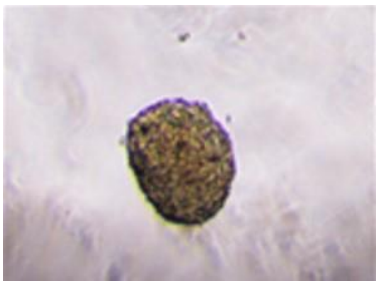

Fig. 2. 3D cell culture of iGL cell. Phase contrast microscope, mag. 40x

3.2. Microscopic observation results of immunofluorescence staining of iGL cell line 2D

Fluorescence microscopy revealed the distribution and localization of the target proteins within the iGL cells. The cells showed positive staining for the target biomolecule, indicating its presence within the cells. This suggests that the immunofluorescence staining technique successfully identified the target biomolecule in the iGL cell line. Representative images show the expression patterns of the markers in the 2D culture condition (Fig. 3). The fluorescence signal was observed in the cytoplasm of the iGL cells, confirming the presence and localization of the target biomolecule.

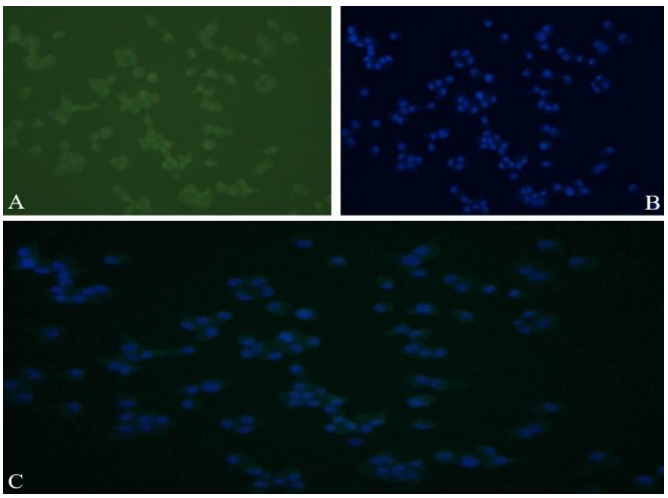

Fig. 3. Immunofluorescence staining of iGL cell line 2D. (A) Cx-36-FITC (green panel), (B) DAPI (blue panel), (C) merged

3.3. Microscopic observation results of immunofluorescence staining of iGL cell line 3D

In the 3D culture condition, immunofluorescence staining revealed the spatial organization and distribution of the target biomolecule within the iGL cells. The fluorescence signal was observed throughout the 3D stru

ctur e, indicating widespread expression of the target biomolecule. This suggests that the immunofluorescence staining technique successfully identified and localized the target biomolecule in both 2D and 3D culture conditions, providing valuable insights into its spatial distribution within the iGL cell line. Fluorescence microscopy revealed the distribution and localization of the target proteins within the iGL cells. Images depict the spatial organization and morphology of iGL cells within the 3D matrix. Representative images show the expression patterns of the markers in the 3D culture condition (Fig. 4).

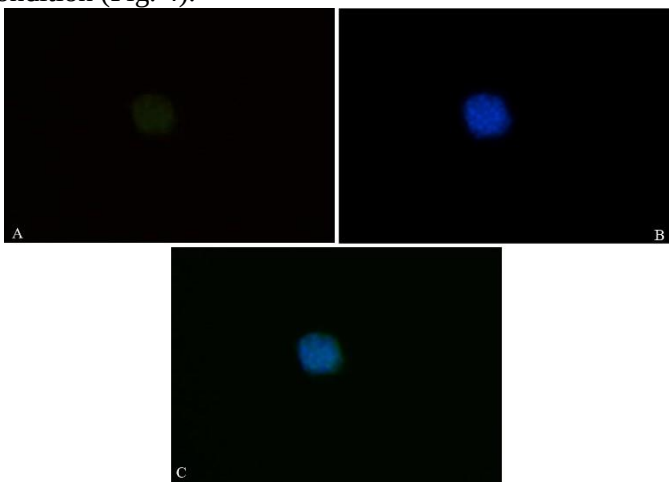

Fig. 4. Immunofluorescence staining of iGL cell line 3D. (A) Cx-36-FITC (green panel), (B) DAPI (blue panel), (C) merged

4. Discussion

The iGL cells, in either a monolayer or 3D culture system, were growing well until two days of incubation. This is in accordance with previous research by (11) that showed iGL cells with a stable morphology and viability

could be derived from 50-100 cells / µL with two days of incubation. The stable morphology and viability are both critical aspects when considering an immunofluorescence assay. Cell morphology may provide valuable information about the shape, size, and organization of the cell. On the other hand, cell viability proposes the proportion of live and healthy cells within in a population (12).

The distinctive aspect of our study is that the cells are different. The iGL cells used in this study are still new and there are not yet many references using them as a research subject, so it is hoped that this protocol study can serve as an additional reference for knowledge regarding iGL cells, especially regarding methods/protocols for developing these iGL cells.

In the blocking section, the materials used are different. We use BSA, while in the literature normal serum/skim milk is used. The reason BSA is used is that it is less expensive than normal serum/skim milk and can still produce a fairly good fluorescent image.

In the antigen retrieval section, sodium citrate is normally used, but now we use a permeable solution (0.3% Triton X-100

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in PBS. To prepare 100 mL: add 300 µl Triton X-100 to 100 mL PBS and mix.)

The similarity of our study to others is that we use viable 3D cell culture samples, that are fixated right away before immunostaining. We also use the same buffer, formaldehyde 10% and antibody dilution. (2)

The advantage of the method in this study is that we use inexpensive, easier materials and goods that are easy to obtain, while the disadvantage of this study is that this method cannot be directly compared with existing methods due to limited studies to date about iGL cells.

For microscopic observation of immunofluorescence staining of iGL cell line in 2D and 3D, images were captured using appropriate filter sets for each fluorophore (FITC for green, DAPI for blue).

To perform immunofluorescence staining on the IGL cell line in an affordable and simple setting, certain tips can be helpful:

1. Utilize cost-effective alternatives for reagents and materials without compromising the quality of the results.

2. Consider optimizing the staining protocol by adjusting the concentrations of antibodies and reagents to minimize wastage.

3. Explore using alternative mounting media and blocking buffers that are affordable and readily available.

4. Take advantage of economical options for fluorescent secondary antibodies without compromising the specificity of the staining.

In addition to the cost-effective alternatives mentioned above, there are further options for conducting immunofluorescence staining on a limited budget. For example, consider exploring local suppliers for discounted reagents and materials, and collaborating with other research groups to share resources and reduce costs. Additionally, optimizing the use of equipment and recycling certain consumables can contribute to cost savings without compromising the quality of the staining results. By leveraging these low-budget options and effectively managing resources, it is possible to achieve successful immunofluorescence staining outcomes while working within budget constraints. By continuing to prioritize meticulous technique and attention to detail, we will be able to generate valuable immunofluorescence data for research on the iGL cell line.

Immunofluorescence staining on the iGL cell line is a valuable technique for visualizing the distribution and localization of target biomolecules. With careful consideration of cost-effective strategies and an understanding of the unique characteristics of the iGL cell line, it is possible to achieve reliable and informative immunofluorescence results even with budget constraints.

Ethical Statement

The ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine - Universitas Prof. Dr. HAMKA on June 18, 2021, with the approval number KEPKK/FK/003/07/2021.

Conflict of interest

The authors declare that there is no conflict of interest was declared by the authors.

Funding

This research was supported by a grant from Faculty of Medicine - Universitas Prof. Dr. HAMKA between 20192020. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

The authors would like to thank all supporting staff at the Faculty of Medicine.

Authors' contributions

Concept: E.N.S., S.S.N., Z.N., N.D.P., Design: E.N.S., S.S.N., Z.N., N.D.P., Data Collection or Processing: E.N.S., S.S.N., N.D.P., Analysis or Interpretation: E.N.S., S.S.N., N.D.P., Literature Search: T.Y., W.S., Writing: E.N.S., T.Y., W.S.

References

1. Cheng R, Zhang F, Li M, Wo X, Su YW, Wang W. Influence of Fixation and Permeabilization on the Mass Density of Single Cells: A Surface Plasmon Resonance Imaging Study. Front Chem. 2019;7:1.
2. Bhattacharyya D, Hammond AT, Glick BS. High-Quality Immunofluorescence of Cultured Cells. 2010,p. 403–410.
3. Maity B, Sheff D, Fisher RA. Immunostaining. 2013, p. 81–105.
4. Barzowska A, Pucelik B, Pustelny K, Matsuda A, Martyniak A, Stępniewski J, et al. DYRK1A Kinase Inhibitors Promote β-Cell Survival and Insulin Homeostasis. Cells. 2021;10(9):2263.

5. Davies MN, O'Callaghan BL, Towle HC. Glucose Activates ChREBP by Increasing Its Rate of Nuclear Entry and Relieving Repression of Its Transcriptional Activity. JBC. 2008; 283(35):24029–24038.

6. Nierode G, Kwon PS, Dordick JS, Kwon SJ. Cell-Based Assay Design for High-Content Screening of Drug Candidates. J MICROBIOL BIOTECHN. 2016; 26(2):213–225.

7. . Liang Y, Jetton TL, Zimmerman EC, Najafi H, Berner DK, Matschinsky FM, et al. Effects of Glucose on Insulin Secretion, Glucokinase Activity, and Transgene Expression in Transgenic Mouse Islets Containing an Upstream Glucokinase PromoterHuman Growth Hormone Fusion Gene. Diabetes. 1994;43(9):1138–1145.

8. Damalanka VC, Han Z, Karmakar P, O'Donoghue AJ, La Greca F, Kim T, et al. Discovery of Selective Matriptase and Hepsin Serine Protease Inhibitors: Useful Chemical Tools for Cancer Cell Biology. J Med Chem. 2019;62(2):480–490.

9. Tseng SC, Hatchell D, Tierney N, Huang AJ, Sun TT. Expression of specific keratin markers by rabbit corneal, conjunctival, and esophageal epithelia during vitamin A deficiency. JCB.1984;99(6):2279–2286.

10. Riley JK, Carayannopoulos MO, Wyman AH, Chi M, Moley KH. Phosphatidylinositol 3-Kinase Activity Is Critical for Glucose Metabolism and Embryo Survival in Murine Blastocysts. JBC. 2006;281(9):6010–6019.

11. Ningsih SS, Avissa R, Stujanna EN, Listyaningsih E, Yashiro T, Sukarya WS. Evaluation of morphology and viability of spheroid derived from Insulin-GLase cell line: A model system to understand Type 2 Diabetes Mellitus. JECM. 2021;38(3):211-215.

12. Sirenko O, Mitlo T, Hesley J, Luke S, Owens W, Cromwell EF. High-Content Assays for Characterizing the Viability and Morphology of 3D Cancer Spheroid Cultures. ASSAY DRUG DEV TECHN. 2015;13(7):402–414.

2. Bukti Konfirmasi Review dan Hasil Review Pertama (29 Maret 2024)

From: Adem Kocaman
To: Sri Ningsih
Subject: Journal of Experimental and Clinical Medicine - Article editing request received
Date: March 29, 2024 at 11:45:13 AM

Dear Sri Ningsih,

The editor requested editing for your article titled 1450725-"Affordable and Simple Protocol for Immunofluorescence Staining of Insulin Secreting iGL Cell Line 2D and 3D".
After completing the operations such as uploading files and replying to sent messages, if any, you can finalize your process with the "Complete Resubmission" button.

Please submit main manuscript as a word file. And figures should be uploaded as jpeg or png file format.

You can reach the article process from the https://dergipark.org.tr/en/journal/1099/article/1450725/author/overview link.

DergiPark

3. Bukti Konfirmasi Submit Revisi Pertama, Respon kepada Reviewer, dan Artikel yang Diresubmit

(29 Maret 2024 - 4 April 2024)

April 4, 2024 at 8:43:18 AM
Article resubmission has been completed by author.
April 4, 2024 at 8:42:41 AM
New article file (Fig 4. Immunofluorescence staining of iGL cell line 3D (3).jpeg) has been uploaded by Sri Ningsih.
April 4, 2024 at 8:41:49 AM
New article file (Fig 4. Immunofluorescence staining of iGL cell line 3D (2).jpeg) has been uploaded by Sri Ningsih.
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Article file (2. Title page.pdf) has been changed by Sri Ningsih.
April 4, 2024 at 8:28:54 AM
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April 4, 2024 at 8:27:33 AM
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April 4, 2024 at 7:55:47 AM
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April 4, 2024 at 7:42:21 AM
Article file (1. Full text.docx) has been changed by Sri Ningsih.
April 3, 2024 at 11:47:25 AM
Adem Kocaman extended the author's period for 15 days.
March 29, 2024 at 11:45:13 AM
Adem Kocaman sent the article to the author for resubmission.

4. Bukti Konfirmasi Review dan Hasil Review kedua (9 Mei 2024)

From: Adem Kocaman
To: Sri Ningsih
Subject: Journal of Experimental and Clinical Medicine - Minor decision marked for the article
Date: May 9, 2024 at 3:01:02 PM

Dear Sri Ningsih,

1450725-"Affordable and Simple Protocol for Immunofluorescence Staining of Insulin Secreting iGL Cell Line 2D and 3D" is the minor decision marked by Adem Kocaman for the article.

Dear Sri Ningsih,

Decision: Minor Revision

Reviewer Reviews

Reviewer-1

Review file-1 uploaded by the reviewer

Comments and Suggestions for Author: Dear author, there are some problems with the grammar. If this work is your original work, if you have found a new method, you need to indicate this in the article. As a reader, I cannot understand whether you are already testing a method or whether you have found a new method. If it is a new method, it would be necessary to state its pros and cons in the discussion section with those used in the literature. But if it is not a new method and this protocol is already known and easily accessible, it is written in great details; as if an assay kit protocol. Yours sincerely

Recommendation: Minor Revision

Reviewer-2

Comments and Suggestions for Author: Firstly, the brand or catalog numbers of some chemicals used in the study are not included (FBS, pyruvic acid, etc). Details of some chemicals were repeated many times (PBS x5 times). There are also some sentences; "incubate for 2 hours at room temperature in the dark. After incubate for 2 hours at room temperature in the dark," ?? While one figure has a scale bar, the others do not. On the other hand, almost all of the figures have low resolution. Images should be presented more carefully. Please check the image resolutions and re-upload. In the 'Materials and methods' section, some sentences are written like a protocol ('Prepare', 'do it carefully'). It is written both like a method (passive voice) and like a protocol. It is a step-by-step protocol or methodology? Please review your sentences. Some prepositions are lack and English must be improved in your manuscript. Language should be checked by an expert. The discussion should be revised according to the results of the study by comparing it more comprehensively with the literature.

Recommendation: Major Revision

The article is in "Under Revision" status. You can reach the process page from the https://dergipark.org.tr/en/journal/1099/article/1450725/author/decision link.

DergiPark

5.Bukti Konfirmasi Submit Revisi Kedua, Respon kepada Reviewer, dan Artikel yang Diresubmit (9 Mei 2024 - 29 Juni 2024)

- Timeline

June 29, 2024 at 5:19:53 AM
Article revision has been completed by author.
June 24, 2024 at 10:16:04 AM
Adem Kocaman extended the author's period for 15 days.
June 14, 2024 at 11:14:00 AM
New article file (Response to Reviewer.docx) has been uploaded by Sri Ningsih.
June 14, 2024 at 11:13:01 AM
Article file (Revised Major&Minor Revision Full text manuscript.docx) reviewer visibility has been disabled by Sri Ningsih.
June 14, 2024 at 11:13:01 AM
Article file (Revised Major&Minor Revision Full text manuscript.docx) has been changed by Sri Ningsih.
May 30, 2024 at 10:26:13 AM
Adem Kocaman extended the author's period for 15 days.
May 14, 2024 at 3:48:34 PM
Adem Kocaman extended the author's period for 15 days.
May 11, 2024 at 11:22:44 AM
Article file (Fig 4. Immunofluorescence staining of iGL cell line 3D (3)600.jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:22:44 AM
Article file (Fig 4. Immunofluorescence staining of iGL cell line 3D (3)600.jpeg) has been changed by Sri Ningsih.
May 11, 2024 at 11:22:06 AM
Article file (Fig 4. Immunofluorescence staining of iGL cell line 3D (2)600.jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:22:06 AM
Article file (Fig 4. Immunofluorescence staining of iGL cell line 3D (2)600.jpeg) has been changed by Sri Ningsih.
May 11, 2024 at 11:21:35 AM
Article file (Fig 4. Immunofluorescence staining of iGL cell line 3D (1)600.jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:21:35 AM
Article file (Fig 4. Immunofluorescence staining of iGL cell line 3D (1)600.jpeg) has been changed by Sri Ningsih.
May 11, 2024 at 11:21:13 AM
Article file (Fig 3. Immunofluorescence staining of iGL cell line 2D. (3)600.jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:21:13 AM
Article file (Fig 3. Immunofluorescence staining of iGL cell line 2D. (3)600.jpeg) has been changed by Sri Ningsih.
May 11, 2024 at 11:20:48 AM
Article file (Fig 3. Immunofluorescence staining of iGL cell line 2D. (2)600.jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:20:48 AM
Article file (Fig 3. Immunofluorescence staining of iGL cell line 2D. (2)600.jpeg) has been changed by Sri Ningsih.
May 11, 2024 at 11:20:26 AM
Article file (Fig 3. Immunofluorescence staining of iGL cell line 2D. (1)600.jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:20:26 AM
Article file (Fig 3. Immunofluorescence staining of iGL cell line 2D. (1)600.jpeg) has been changed by Sri Ningsih.

May 11, 2024 at 11:19:25 AM
Article file (Fig 2. 3D cell culture of iGL cell. Phase contrast microscope, mag. 40x600.jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:19:25 AM
Article file (Fig 2. 3D cell culture of iGL cell. Phase contrast microscope, mag. 40x600.jpeg) has been changed by Sri Ningsih.
May 11, 2024 at 11:18:24 AM
Article file (Fig 1. Monolayer cell culture of iGL cell. Phase contrast microscope, mag. 100x (B)600 (1).jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:18:24 AM
Article file (Fig 1. Monolayer cell culture of iGL cell. Phase contrast microscope, mag. 100x (B)600 (1).jpeg) has been changed by Sri Ningsih.
May 11, 2024 at 11:17:54 AM
Article file (Fig 1. Monolayer cell culture of iGL cell. Phase contrast microscope, mag. 40x (A)600.jpeg) reviewer visibility has been disabled by Sri Ningsih.
May 11, 2024 at 11:17:54 AM
Article file (Fig 1. Monolayer cell culture of iGL cell. Phase contrast microscope, mag. 40x (A)600.jpeg) has been changed by Sri Ningsih.
May 9, 2024 at 3:01:02 PM
Adem Kocaman sent the article to the author for revision.

Response to Reviewer Revisi 2

Affordable and Simple Protocol for Immunofluorescence Staining of Insulin Secreting iGL Cell Line 2D and 3D

Reviewer #1

We are grateful to Reviewer #1 for the critical comments and useful suggestions that have helped us to improve our paper. As indicated in the responses, we have taken all these comments and suggestions into account in the revised version of our manuscript.

Dear author, there are some problems with the grammar.

Thank you for your suggestion.
We have done proofreading to improve the grammar in this manuscript.

If this work is your original work, if you have found a new method, you need to indicate this in the article. As a reader, I cannot understand whether you are already testing a method or whether you have found a new method. If it is a new method, it would be necessary to state its pros and cons in the discussion section with those used in the literature. But if it is not a new method and this protocol is already known and easily accessible, it is written in great details; as if an assay kit protocol. Yours sincerely

Thank you for your suggestion.
The distinctive aspect of our study is that the cells are different. The iGL cells used in this study are still new and there are not yet many references using them as a research subject, so it is hoped that this protocol study can serve as an additional reference for knowledge regarding iGL cells, especially regarding methods/protocols for developing these iGL cells.
In the blocking section, the materials used are different. We use BSA, while in the literature normal serum/skim milk is used. The reason BSA is used is that it is less expensive than normal serum/skim milk and can still produce a fairly good fluorescent image.
The pros of this study are that we use inexpensive, easier materials and goods that are easy to obtain, while the cons of this study is that this method cannot be directly compared with existing methods due to limited studies to date about iGL cells.

We already included all the statements above and also stated the pros and cons in the discussion section.

Reviewer #2

We are grateful to Reviewer #2 for the critical comments and useful suggestions that have helped us to improve our paper. As indicated in the responses, we have taken all these comments and suggestions into account in the revised version of our manuscript.

Comments and Suggestions for Author: Firstly, the brand or catalog numbers of some chemicals used in the study are not included (FBS, pyruvic acid, etc).

As you indicated, we have completed the catalog number of materials that we use. We already included it in the materials and methods section.

Details of some chemicals were repeated many times (PBS x5 times). There are also some sentences; "incubate for 2 hours at room temperature in the dark. After incubate for 2 hours at room temperature in the dark," ??

As you indicated, we already revised it.

While one figure has a scale bar, the others do not. On the other hand, almost all of the figures have low resolution. Images should be presented more carefully. Please check the image resolutions and re-upload.

As you indicated, The resolution of the image has been improved.

In the 'Materials and methods' section, some sentences are written like a protocol ('Prepare', 'do it carefully'). It is written both like a method (passive voice) and like a protocol. It is a step-by-step protocol or methodology?

Thank you for your suggestion.
This study provides valuable insights into an affordable and simple protocol for immunofluorescence staining of the iGL cell line in both 2D and 3D culture conditions. We describe cost-effective alternatives for reagents and materials without compromising the quality of the results, such as the use of Bovine Serum Albumin **(**BSA) 3% as a blocking buffer instead of normal serum.

Please review your sentences. Some prepositions are lack and English must be improved in your manuscript. Language should be checked by an expert. The discussion should be revised according to the results of the study by comparing it more comprehensively with the literature.

Thank you for your suggestion.
We have done proofreading to improve the grammar in this manuscript.
We've also improved the manuscript with additional previous literature compare with our result in the discussion section.

6. Bukti Konfirmasi Artikel Accepted (8 Juli 2024)

From: Adem Kocaman
To: Sri Ningsih
Subject: Journal of Experimental and Clinical Medicine - Acceptance decision marked for article
Date: July 8, 2024 at 1:11:30 PM
Dear Sri Ningsih,

1450725-"Affordable and Simple Protocol for Immunofluorescence Staining of Insulin Secreting iGL Cell Line 2D and 3D" is the acceptance decision marked by Adem Kocaman for the article.

Dear Sri Ningsih,

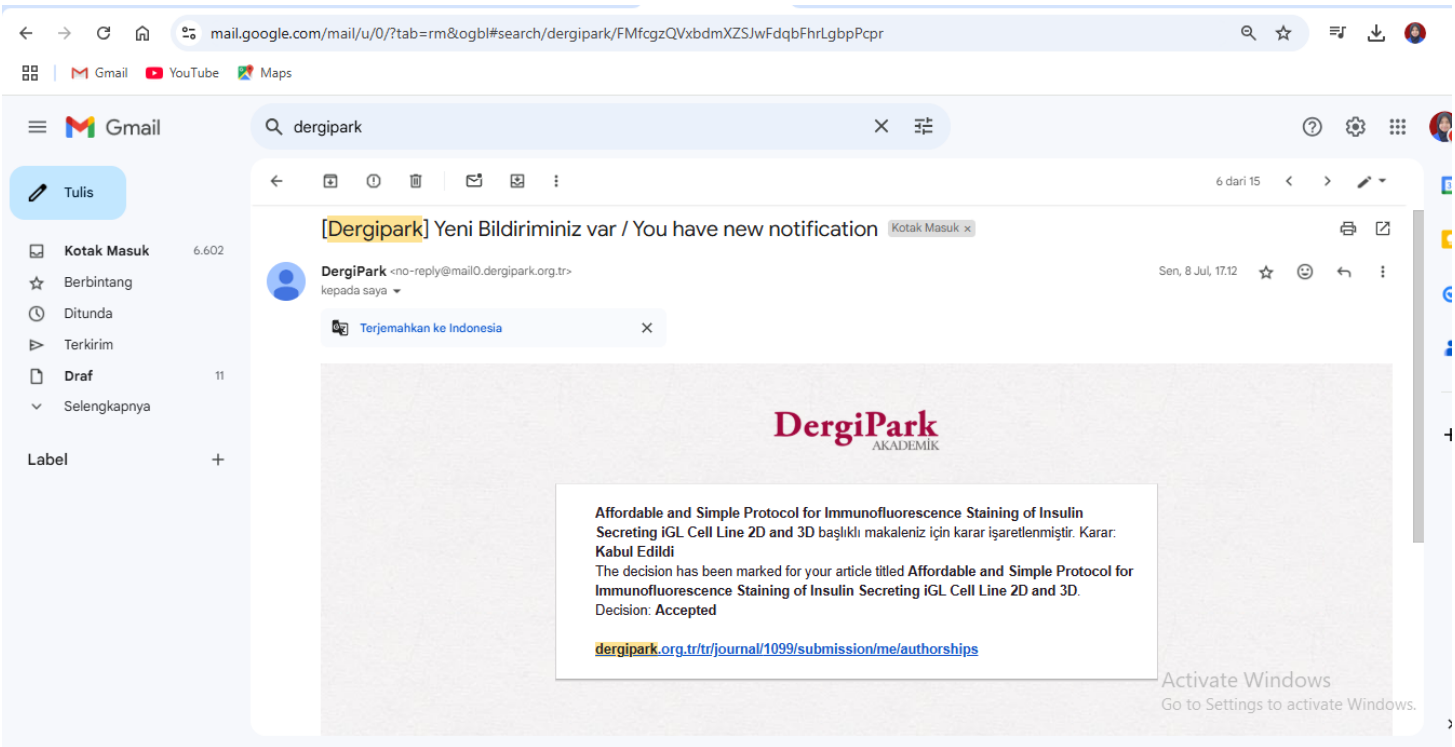
Decision: Accepted

Reviewer Reviews

Reviewer-1

Comments and Suggestions for Author: The authors increased the resolution (dpi) of the figures, but they were still tiny in size. -images may look pixelated (with small pixel dimensions)-

Recommendation: Accepted



7. Bukti Konfirmasi Artikel Published Online (30 Agustus 2024)

From: Büşra Nur Özcan
To: Sri Ningsih
Subject: Journal of Experimental and Clinical Medicine - Your article's workflow has been completed
Date: August 30, 2024 at 5:59:17 PM

Dear Sri Ningsih,

Your article's workflow titled 1450725-"Affordable and Simple Protocol for Immunofluorescence Staining of Insulin Secreting iGL Cell Line 2D and 3D" has been completed. The article is in Ready for an issue.

Notification will be sent when the issue is published. You can learn the publication planning of the journal from the editor.

DergiPark