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NanoFect Transfection Reagent Handbook

For efficient DNA transfection of a broad range of cell lines

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Kit Contents

NanoFect Transfection Reagent	(0.5 ml)	(1 ml)	(4 x 1 ml)
Catalog no.	**301204**	**301205**	**301207**
Transfections per kit (in 24-well plates)	**Up to 250**	**Up to 500**	**Up to 2000**
NanoFect Transfection Reagent	0.5 ml	1 ml	4 x 1 ml
Handbook	1	1	1

Shipping and Storage

NanoFect Transfection Reagent is supplied as a ready-to-use solution and is shipped at ambient temperature without loss in stability. However, it should be stored at 2–8°C upon arrival. It is stable for 1 year at 2–8°C. NanoFect Transfection Reagent does not need to be stored on ice during the transfection procedure.

Product Use Limitations

NanoFect Transfection Reagent is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding NanoFect Transfection Reagent or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of NanoFect Transfection Reagent is tested against predetermined specifications to ensure consistent product quality.

Introduction

NanoFect Transfection Reagent, based on advanced nanopolymer technology, is a chemically synthesized, lipid-free reagent which ensures highly efficient DNA transfection in a broad range of cell types.

Principle and procedure

NanoFect Transfection Reagent uses advanced nanopolymer technology for highly efficient DNA transfection with low cytotoxicity. It is composed of ultra small nanopolymers which form transfection complexes in the nanometer size range when mixed with DNA.

The nanopolymer technology ensures reliable, reproducible performance for transient or stable transfections. It also facilitates flexible handing, such as preparation and storage of transfection complexes prior to transfection. NanoFect Reagent is suited to preclinical studies as well as basic research, and to low- and high-throughput and small- and large-scale transfections. In addition, the absence of lipids makes this reagent suitable for lipid or signal transduction research.

NanoFect Reagent is completely chemically synthesized, free of animal-derived components, and has been tested for the absence of endotoxins. For these reasons, it is ideal for use when absence of animal-derived components is a priority, for example in biopharmaceutical applications. Absence of animal-derived components also facilitates regulatory compliance.

NanoFect Reagent enables transfection in the presence of serum without lowering transfection efficiencies.

Cell-specific QIAGEN transfection protocols

The TransFect Protocol Database is an invaluable resource for transfection experiments. Simply visit www.qiagen.com/TransFect and then enter the cell type, nucleic acid, and plate format to receive a QIAGEN transfection protocol to print out or download in convenient PDF format. Use of the TransFect Protocol Database is free of charge and no registration is required.

Description of protocols

Two protocols are provided in this handbook: the Fast-Forward Protocol and the Traditional Protocol (Figure 1). In the Fast-Forward Protocol, plating and transfection of cells are performed on the same day. DNA is diluted in culture medium without serum. NanoFect Transfection Reagent is added to the diluted DNA to produce NanoFect–DNA complexes. The cells are seeded and then complexes are added directly to the freshly seeded cells.

In the Traditional Protocol, cells are plated 24 hours prior to transfection. Cells are seeded in culture medium containing serum and antibiotics the day before transfection and incubated under normal growth conditions. The next day, DNA is diluted in culture medium without serum. NanoFect Transfection Reagent is added directly to the diluted DNA to produce NanoFect–DNA complexes. During complex formation, the medium on the cells is changed. Next, the complexes are added to the cells.

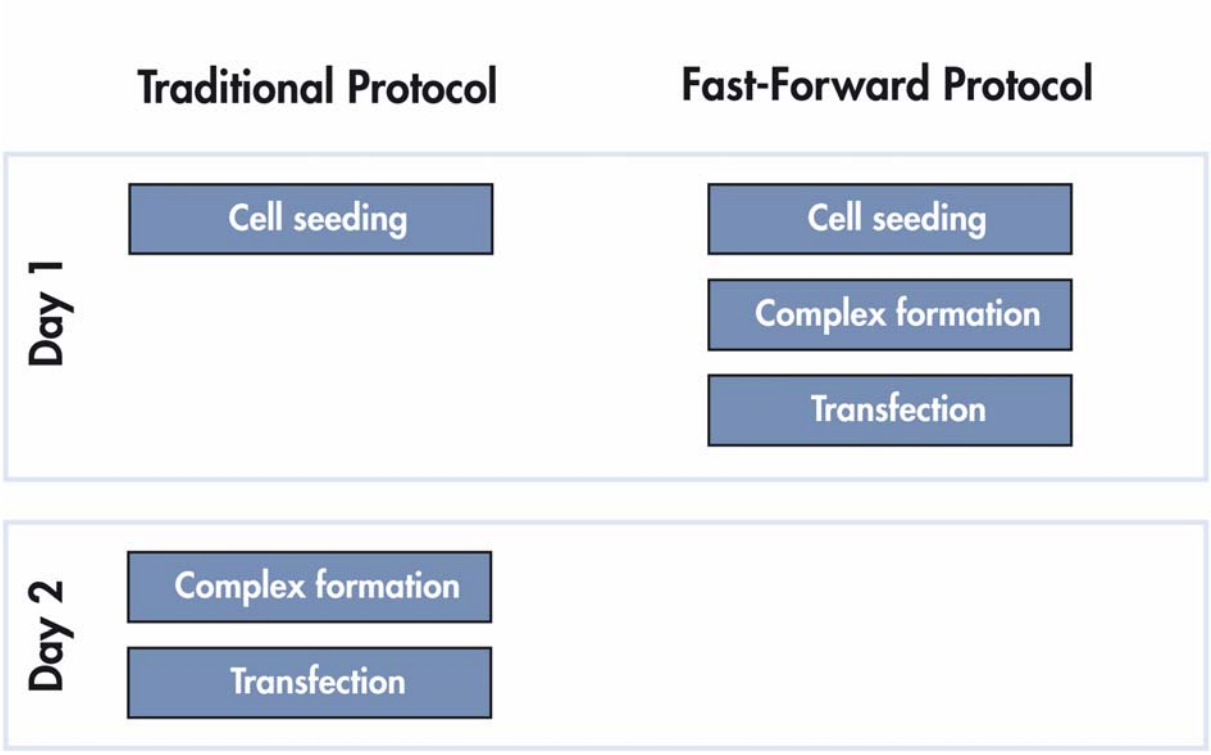

Figure 1. The Fast-Forward Protocol saves time and labor. In the Fast-Forward Protocol, cell seeding, complex formation, and transfection are all performed on the same day. In the Traditional Protocol, cell seeding is performed the day before transfection.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Culture medium
- DNA of interest
- Cells of interest

Important Notes

Optimizing DNA and NanoFect Transfection Reagent amounts

To achieve optimal transfection results for a given cell line/DNA combination, the amount of DNA and NanoFect Reagent should be optimized. Table 1 shows a pipetting scheme for optimizing transfection of cells in 24-well plates. This scheme shows 9 different conditions which we recommend to test when optimizing transfection (i.e., when determining the conditions that provide highest transfection efficiency and/or lowest cytotoxicity). As a starting point, we recommend using 0.75 µg DNA and 2 µl Reagent (in bold in Table 1). For transfection using other culture formats, refer to Tables 3 and 4, pages 12 and 14 respectively.

Table 1. Pipetting scheme for optimizing transfection in a 24-well plate*

Amount of DNA	0.38 µg	0.38 µg	0.38 µg
Volume of NanoFect Reagent	0.67 µl	1 µl	2 µl
Amount of DNA	0.75 µg	**0.75 µg**	0.75 µg
Volume of NanoFect Reagent	1.33 µl	**2 µl**	4 µl
Amount of DNA	1.13 µg	1.13 µg	1.13 µg
Volume of NanoFect Reagent	2 µl	3 µl	6 µl

The recommended starting point for transfection is shown in bold.

* Amounts given are per well of a 24-well plate.

Optimizing transfection in different formats

Tables 3 (page 12) and 4 (page 14) give starting points for optimization of transfection in different formats for the Fast-Forward Protocol and the Traditional Protocol, respectively.

Preparing a master mix for multiple transfections

If performing multiple transfections in parallel, a transfection complex master mix should be prepared for distribution into the plate wells. Prepare 10% more master mix than is required, or at least enough for 1 extra sample, to allow for pipetting errors. Keep the overall amounts and volumes, as given in Tables 3 and 4, constant. Prepare a dilution of the DNA in medium (without serum and antibiotics), add NanoFect Reagent, and continue as indicated in the protocols.

For example, calculate the components of the transfection complex master mix for transfection in 24-well plates as follows:

DNA: (number of replicates+1) x 0.75 µg

Medium: (number of replicates+1) x 60 µl

NanoFect Reagent: (number of replicates+1) x 2 µl

For the Traditional Protocol:

Medium added to the complexes: (number of replicates +1) x 500 µl

Cell density at transfection

The optimal cell confluency for transfection should be determined for every new cell type to be transfected and kept constant in future experiments. This is achieved by counting the cells before seeding and, in the case of the Traditional Protocol, by keeping the interval between seeding and transfection constant. This ensures that cell density is not too high and the cells are in the optimal physiological condition at transfection. For adherent cells, the optimal confluency at the time of complex addition is normally 40–80%.

The recommended number of cells to seed for different formats for the Fast-Forward Protocol and the Traditional Protocol is shown in Table 2. These cell numbers can be used as starting points for transfection optimization. Cell densities recommended for the Fast-Forward Protocol are usually 2–3 times higher than those recommended for the Traditional Protocol. However, the optimal cell number will be different for different cell types; for example small-sized cells, such as HEK 293, should be transfected at higher cell numbers than larger-sized cells.

Table 2. Recommended number of cells per culture vessel

Culture format	Fast-Forward Protocol	Traditional Protocol
96-well plate	1–8 x 10^4	0.5–4 x 10^4
24-well plate	0.4–1.6 x 10^5	2–8 x 10^4
6-well plate	1.6–8 x 10^5	0.8–4 x 10^5
100 mm dish	1–5 x 10^6	0.5–2.5 x 10^6

Protocol: Fast-Forward Transfection of Cells with DNA in 24-Well Plates

The following protocol is provided as a starting point for optimization of transfection of cells in 24-well plates without preplating of cells 24 h prior to transfection. The amounts given are for one well of a 24-well plate. For transfection using other culture formats, refer to Table 3, page 12.

Procedure

1. **Dilute 0.75 µg DNA dissolved in TE buffer, pH 7–8 (minimum DNA concentration: 0.1 µg/µl) with medium without serum, proteins, or antibiotics, to a total volume of 60 µl.**

   For example, if the DNA concentration is 1 µg/µl, dilute 0.75 µl DNA in 59.25 µl medium.

2. **Add 2 µl NanoFect Transfection Reagent. Mix by pipetting up and down or vortexing. Centrifuge for a few seconds to remove any liquid from the top of the tube if necessary.**

3. **Incubate the samples for 10–15 min at room temperature (15–25°C) to allow transfection complex formation. Continue with steps 4 and 5 during the incubation time.**

   Note: Transfection complex formation takes a minimum of 10–15 min. The transfection complexes will remain stable during the time it takes to prepare the cells for transfection. However, the incubation time should not be extended for longer than is necessary for cell preparation.

4. **Harvest the cells by trypsinization and suspend in culture medium containing serum and antibiotics.**

   Note: The cells should be healthy and in logarithmic growth phase.

   It is important that serum and antibiotics are present in the culture medium at this point because transfections are performed without changing the medium. The cultivation of cells over this time without serum would deprive the cells of essential growth factors. This does not apply to cells that are routinely cultivated without serum.

5. **Count the cells in the harvested cell suspension and adjust the cell density to 0.4–1.6 x 10^5 cells in 500 µl (depending on the cell line). The optimal cell density should be determined for each cell line.**

   Note: For example, cell density should be adjusted to 0.8–3.2 x 10^5 cells per ml for a final cell density at transfection of 0.4–1.6 x 10^5 cells in 500 µl.

   As plating and transfection of cells are carried out on the same day, higher cell densities are required than would be necessary if the cells had a longer incubation time prior to transfection. As a guideline, the required cell

number is usually 2–3 fold higher than that used for the Traditional Protocol (where cells are plated on the day before transfection).

6. **Add 500 µl of the cell suspension to a well of a 24-well plate. Next, add the transfection complexes to the well. Mix by pipetting up and down twice.**
7. **Incubate the cells with the transfection complexes under their normal growth conditions (typically 37°C and 5% CO_2).**

   In most cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the complexes after 6–18 hours and add fresh culture medium.
8. **Assay the cells for expression of the transfected gene after an appropriate incubation time. The length of the incubation time depends on the assay and the transfected gene.**
9. **For stable transfections, passage the cells into the appropriate selection medium 24–48 h after transfection. Maintain the cells in selective culture medium until colonies appear.**

Table 3. Starting points for transfection in different formats using the Fast-Forward Protocol

Culture format	DNA amount (µg)	Final volume of diluted DNA (µl)	NanoFect Transfection Reagent (µl)	Volume of cell suspension (µl)
Protocol step	1	1	2	6
96-well plate	0.25	50	0.5	100
24-well plate	0.75	60	2	500
6-well plate	2	100	6	2000
100 mm dish	7	300	19	10,000

Protocol: Transfection of Cells with DNA in 24-Well Plates (Traditional Protocol)

The following protocol is provided as a starting point for optimization of transfection of cells in 24-well plates. In this protocol, cells are plated 24 h prior to transfection. The amounts given are for one well of a 24-well plate. For transfection using other culture formats, refer to Table 4, page 14. This protocol can be used for sensitive cell lines.

Procedure

1. **The day before transfection, seed 2–8 x 10^4 cells (depending on the cell type) in 500 µl of an appropriate culture medium containing serum and antibiotics.**
2. **Incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).**

   Cells should be 40–80% confluent on the day of transfection.
3. **On the day of transfection, dilute 0.75 µg DNA dissolved in TE buffer, pH 7–8 (minimum DNA concentration: 0.1 µg/µl) with medium without serum, proteins, or antibiotics to a total volume of 60 µl.**
4. **Add 2 µl NanoFect Transfection Reagent to the DNA solution. Mix by pipetting up and down or vortexing. Centrifuge for a few seconds to remove any liquid from the top of the tube if necessary.**
5. **Incubate the samples for 10–15 min at room temperature (15–25°C) to allow complex formation.**
6. **While complex formation takes place, gently aspirate the medium from the cells and add 500 µl fresh medium (containing serum and antibiotics) to the cells.**
7. **Add the transfection complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**
8. **Incubate the cells under their normal growth conditions and analyze the cells after an appropriate time.**

   In most cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the complexes after 6–18 hours and add fresh culture medium.
9. **For stable transfections, passage the cells into the appropriate selection medium 24–48 h after transfection. Maintain the cells in selective culture medium until colonies appear.**

Table 4. Starting points for transfection in different formats using the Traditional Protocol

Culture format	DNA amount (µg)	Final volume of diluted DNA (µl)	NanoFect Reagent (µl)	Volume of medium* added to cells (µl)
Protocol step	3	3	4	6
96-well plate	0.25	50	0.5	100
24-well plate	0.75	60	2	500
6-well plate	2	100	6	2000
100 mm dish	7	300	19	10,000

* Culture medium containing serum and antibiotics.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Low transfection efficiency	
a) Suboptimal reagent to DNA ratio	If the ratio of transfection reagent to DNA is suboptimal, the overall charge of the complexes may be negative, neutral, or strongly positive, which can lead to inefficient adsorption to the cell surface. Optimize the NanoFect Transfection Reagent to DNA ratio using Table 1, page 9.
b) Insufficient NanoFect Reagent–DNA complex	If the transfection efficiency is lower than expected and cytotoxicity is acceptably low, increase the overall amount of NanoFect Reagent–DNA complex added to the cells. See the pipetting scheme in Table 1, page 9.
c) Suboptimal incubation time	Different cell types achieve maximal expression levels at different times after transfection. This should be kept in mind when determining the length of incubation after transfection. If the time point of maximal expression is not known for a particular cell line, a time-course experiment may be necessary.
d) Suboptimal cell density	If cell density at the time of adding NanoFect Reagent–DNA complexes is not at an optimal level, this can lead to insufficient uptake of complexes into the cells. For adherent cells, the optimal confluency for DNA transfection is normally 40–80%. Be sure to seed cells a minimum of 24 h before transfection if using the Traditional Protocol. Usually higher cell numbers are required for the Fast-Forward Protocol in order to compensate for the reduced doubling time between plating and transfection.

	Comments and suggestions
e) Vector influence	Factors such as the promoter, origin of replication, and plasmid size influence gene expression rate. The optimal quantity of plasmid DNA used for transfection is dependent on the expression rate of the plasmid.
f) Poor DNA quality	DNA should be of high quality, as impurities can lower transfection efficiency. Plasmid DNA should be purified using HiSpeed®, QIAfilter, or QIAGEN Plasmid Kits. For endotoxin-sensitive cells, we recommend using EndoFree® Plasmid Kits (see ordering information, page 20).
Excessive cell death	
a) Excessive exposure of cells to NanoFect Reagent–DNA complexes	If sensitive cells demonstrate extensive cell death after treatment with NanoFect Reagent–DNA complexes, remove the complexes after 6 h and add fresh medium.
b) Concentration of NanoFect Reagent–DNA complexes too high	Decrease the amount of NanoFect Reagent–DNA complexes added to cells, but keep the ratio of NanoFect Reagent to DNA constant.
c) Cells are stressed	Avoid stressing cells with temperature shifts and long periods without medium during washing steps. Ensure that cell density is not too low at transfection. For adherent cells, the optimal confluency for transfection is 40–80%. Be sure to seed cells a minimum of 24 h before transfection if using the Traditional Protocol. Usually higher cell numbers are required for the Fast-Forward Protocol in order to compensate for the reduced doubling time between plating and transfection. We recommend performing the transfection in the presence of serum so that the cells are not deprived of necessary growth factors and nutrients during this long incubation.

	Comments and suggestions
d) Poor DNA quality	DNA should be of high quality, as impurities can lower transfection efficiency. Plasmid DNA should be purified using HiSpeed, QIAfilter, or QIAGEN Plasmid Kits. For endotoxin-sensitive cells, we recommend using EndoFree Plasmid Kits (see ordering information, page 20).

Variable transfection efficiencies in replicate experiments

a) Inconsistent cell confluencies in replicate experiments	Count cells before seeding to ensure that the same number of cells is seeded for each experiment. Keep incubation times between replicate seeding and complex addition consistent between experiments. Cells should be seeded at least 24 h before transfection if using the Traditional Protocol.
b) Possible mycoplasma contamination	Mycoplasma contamination influences transfection efficiency. Variations in the growth behavior of mycoplasma-infected cells will lead to different transfection efficiencies between replicate experiments.
c) Cells have been passaged too many times	Cells that have been passaged a large number of times tend to change their growth behavior, morphology, and potential for transfection. When cells with high passage numbers are used for replicate experiments, decreased transfection efficiencies may be observed in later experiments. We recommend using cells with a low passage number (<50 splitting cycles).
d) Serum variability	Variations in serum quality can lead to differences in transfection efficiency. It is advisable to test a small lot of serum from a reputable supplier in a control experiment. Once a given lot has yielded satisfactory and reproducible results, sera from this lot should be used for further experiments.

Difficulty isolating cell clones stably expressing DNA

a) Suboptimal concentration of the selection reagent	The optimal concentration of a given selection reagent is dependent on the cell density and the cell type. This should be evaluated for each cell type.

	Comments and suggestions
b) Suboptimal incubation time between transfection and selection	Ensure that the incubation time between transfection and the start of selection is long enough to allow optimal expression of the resistance gene used for selection.
c) Culture consists of a cell mixture	Ensure that the clones consist of single cells stably expressing DNA and not a mixture of stably expressing clones and resistant clones which do not express the DNA. The latter case may lead to loss of stably expressing cells over time.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
NanoFect Transfection Reagent (0.5 ml)	NanoFect Transfection Reagent for up to 250 transfections in 24-well plates	301204
NanoFect Transfection Reagent (1 ml)	NanoFect Transfection Reagent for up to 500 transfections in 24-well plates	301205
NanoFect Transfection Reagent (4 x 1 ml)	NanoFect Transfection Reagent for up to 2000 transfections in 24-well plates	301207
Accessories		
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QIAGEN Plasmid Mini Kit (25)*	25 QIAGEN-tip 20, Reagents, Buffers	12123
EndoFree Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, 10 QIAfilter Maxi Cartridges, Endotoxin-free Buffers	12362

* Larger prep and kit sizes available. Find out more at www.qiagen.com .

Notes

Notes

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the NanoFect Transfection Reagent to the following terms:

1. The NanoFect Transfection Reagent may be used solely in accordance with the NanoFect Transfection Reagent Handbook and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the NanoFect Transfection Reagent Handbook and additional protocols available at www.qiagen.com .
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