Gentle AP30 Nucleic Acid Transfection Reagent for Primary Cells

Gentle AP30

Instruction Manual

Version 1.3

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01 / Product Overview

Peptide-based nucleic acid delivery is a state-of-the-art delivery technology that can significantly improve nucleic acid transfection of a wide range of cells, including primary cells and hard-to-transfect cell lines [1][2]. Based on the high-throughput peptide screening platform, GentleFect™ technology accurately identifies endogenous peptide sequences with phase separation potential, and developed the Gentle AP30 nucleic acid transfection reagent for primary cells. Peptides are self-assembled by liquid-liquid phase separation with nucleic acids to form nano-scale droplets, which can be efficiently internalized with the help of cell membrane pinocytosis, resulting significantly improvement of the nucleic acid delivery efficiency of cells across all species, while cell viability are also maintained.

02 / Product Features

- The product is suitable for transfection of various nucleic acid molecules, including DNA, mRNA, siRNA, circRNA, Crispr, etc.;
- The product is a ready-to use transfection reagent, easily add nucleic acids to the reagent to configure the transfection solution in a single step.

03 / Product Specifications

Catalog number	Name	Size
1010001	Gentle AP30 Nucleic Acid Transfection Reagents for Primary Cells	3.6 mL
1010002	Gentle AP30 Nucleic Acid Transfection Reagents for Primary Cells	15.0 mL
1010003	Gentle AP30 Nucleic Acid Transfection Reagents for Primary Cells	40.0 mL

04 / Storage

4°C for short storage and -20°C for long storage.

05 / Product Components

Gentle AP30 Nucleic Acid Transfection Reagents for primary cells

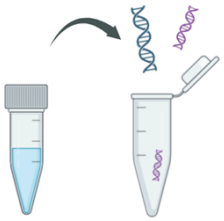
06 / Preparation

Adherent cells (cell confluence rate of 70-80%) or suspension cells, ensure cell viability of > 90%, enzyme-free EP tubes. Restore the complete medium, Opti-MEM medium, trypsin, and nucleic acid transfection reagent to room temperature, and keep the nucleic acids cold, **vortex transfection reagents prior to use**. If the nucleic acid is in a powder state, dissolve it in DNase/RNase-Free water in advance and mix thoroughly, all these procedures should be performed in a clean bench.

07-1 / Experimental Procedure (96 well. Suspension-based Procedure)

1 Transfection mix preparation:

- **Mix Gentle AP30 transfection reagent thoroughly before use**, For each 96-well, add 40 µL transfection reagent to a 1.5 mL Nuclease free centrifuge tube, then add 0.5–1 µg nucleic acids, **gently pipette for 30–60 s**, and incubate at room temperature for at least 3 minutes.



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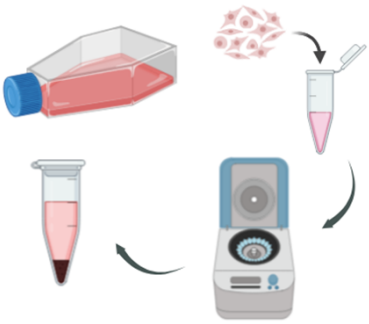


2 Cell sample preparation:

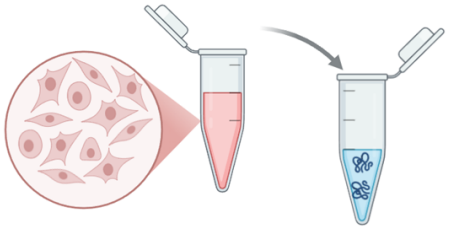
- **Suspension cells** were harvested by centrifugation at 300g for 5 minutes. Remove cell supernatant, and wash with **FBS-free Opti-MEM.** After a second centrifugation at 300g for 5 minutes, discard cell supernatant, and resuspend in Opti-MEM to **a final density of 5×10^6 cells/mL**.
- **Adherent cells** were harvested by washing with 1x PBS, followed by trypsinization. The cells were then centrifuged at 300g for 5 minutes, remove cell supernatant, and wash with **FBS-free Opti-MEM**, centrifuged again at 300g for 5 minutes, discard cell supernatant, and resuspend in Opti-MEM **to a final density of 1×10^6 cells/mL.**

(Note: Maintain cell viability >90%. Remove complete medium to avoid interference from FBS or proteins, substitute with PBS when Opti-MEM is unavailable.)

3 Cell transfection:

- Add 20 µL of cell suspension to the transfection mix in 1.5 mL tube, and mix gently. Incubate at 37 °C for 30 min.

(Note: For scale-up transfections, maintain the ratio of transfection reagent to cell suspension at 2:1(v/v) and mix thoroughly in larger size tubes. Incubation time should be optimized according to cell status, **do not exceed 2 h; prolonged incubation may induce cytotoxicity**.)



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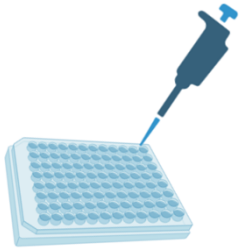


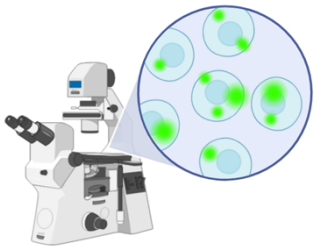
4 Transfect termination:

- To terminate the transfection, add 1 mL of complete medium to the cells, followed by centrifugation at 300g for 5 minutes. Carefully discard the supernatant, **retain 20–30 µL of residual medium to avoid cell loss.** Resuspend the pellet in 200 µL complete medium, transfer to the plate for continued culture.

(Note: When scaling up transfection, terminate with complete medium ≥2× the transfection mixture volume, replenish with the standard volume of complete medium.)

5 Result:

- Detect target gene expression at 12–48 h post-transfection.



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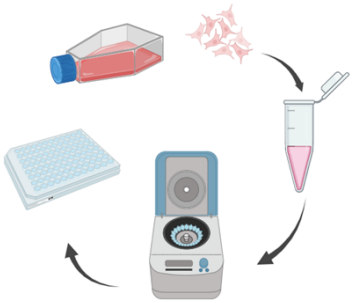
07-2 / Experimental Procedure (96 well. Adherent-cell Procedure)

1 Cell sample preparation:

- Cells were passaged and seeded 24 h before transfection **to reach 70–90 % confluency at the time of transfection**. **(Note: Cell viability >90 % is required to ensure maximal transfection efficiency.)**



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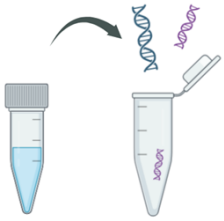

2 Transfection mix preparation:

- **Mix Gentle AP30 transfection reagent thoroughly before use**, For each 96-well, add 40 µL transfection reagent to a 1.5 mL Nuclease free centrifuge tube, then add 0.5–1 µg nucleic acids, **gently pipette for 30–60 s**, and incubate at room temperature for at least 3 minutes.

3 Cell transfection:

- Discard 96-well cell supernatant, **and rinse once or twice with serum-free Opti-MEM.** **(Note: complete medium must be removed to prevent interference from FBS or proteins; PBS can be substituted if Opti-MEM is not available.)**
- Add 20 µL Opti-MEM to the 96-well, then **slowly add** 40 µL prepared transfection complex. **Gently shake the 96-well plate to distribute the liquid evenly.** **(Note: Maintain a 2:1 (v/v) ratio of transfection complex to Opti-MEM.)**
- Incubate at 37 °C for 30 minutes. (**Note:** Incubation time should be optimized according to cell status**, do not exceed 2 h; prolonged incubation may induce cytotoxicity**..)

(Note: For scale-up transfections, increase reagent volumes proportionally according to the recommended ratio.)

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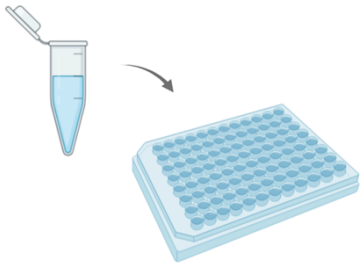

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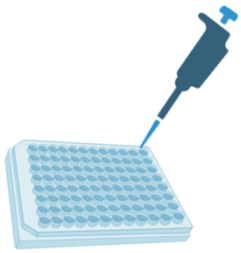


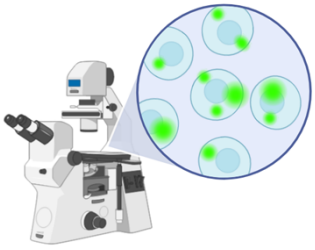
4 Transfect termination:

After incubation, add 100 µL of complete medium to terminate transfection, then gently remove the supernatant. Add 150–200 µL of fresh complete medium to the plate, then incubate at 37 °C with 5% CO_2 for continued culture.

(Note: When scaling up transfection, terminate with complete medium ≥2× the transfection mixture volume, replenish with the standard volume of complete medium.)

5 Result:

- Detect target gene expression at 12–48 h post-transfection.



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08 / Transfection System

Table 1. DNA/mRNA transfection amounts

Plate format	AP30 (μL)	DNA/mRNA (μg)	Cell suspension (μL)	Suspension cells Number of cells	Adherent cells Number of cells
96 wells	40	0.5-1	20	1 x10^5	2 x10^4
48 wells	80	1-2	40	2x10^5	4 x10^4
24 wells	200	2.5-5	100	5 x10^5	1 x10^5
12 wells	500	7.5-15	250	1 x10^6	2.5 x10^5
6 wells	800	10-20	400	2 x10^6	4 x10^5

Table 2. siRNA transfection amounts

Plate format	AP30 (μL)	siRNA (pmol)	Cell suspension (μL)	Suspension cells Number of cells	Adherent cells Number of cells
96 wells	40	40	20	1 x10^5	2 x10^4
48 wells	80	80	40	2x10^5	4 x10^4
24 wells	200	200	100	5 x10^5	1 x10^5
12 wells	500	600	250	1 x10^6	2.5 x10^5
6 wells	800	800	400	1 x10^6	4 x10^5

① Incubate the transfection complex with cells, maintain a 2:1 (v/v) ratio of transfection reagent to cell suspension; for adherent cells, dilute the mixture to the required volume with serum-free medium.

② In co-transfection experiments, the total nucleic acid dose per well equals the combined amount of all constructs; for 96-well plates, the cumulative nucleic acid load should be 0.5–1 μg per well.

09 / Frequently Asked Questions

- **What are the differences in transfection procedure for adherent vs. suspension cells?**

  Suspension cells are cultured in suspension, allowing for a higher cell density per well compared to adherent cells. Both types of cells can be transfected during cell passaging, so adherent cells do not need to be plated before use. In addition, with numbers of experiments tested, transfect cell suspension mixture in an EP tube help maximize the transfection efficiency.

- **How to determine the amount of transfection reagent?**

  It is recommended to follow the transfection reagent amounts specified in Tables 1 and 2. Alternatively, the amount of transfection reagent can be adjusted proportionally according to the number of cells, also suspension cells can be transfected according to the common density used in customer's cell culture system.

- **How to improve transfection efficiency?**

  ① **Optimize Cell Condition**: Prior to transfection, ensure cells viability exceed 90%. This can be assessed using standard viability assays such as trypan blue exclusion or propidium iodide staining.

  ② **Adjust Reagent Concentrations:** Incrementally increase the amounts of transfection reagent and nucleic acid to identify the optimal ratio that maximizes transfection efficiency without compromising cell viability.

  ③ **Optimize Incubation Time:** Experiment with varying incubation times to determine the ideal duration for your specific cell type. Both prolonged and shortened incubation periods can influence transfection efficiency and cell viability, so it is essential to find a balance that yields the best results.

- **What factors affect transfection efficiency?**
  ① Do not use medium containing FBS during cell transfection, FBS will significantly compromise the efficiency of cell transfection;
  ② Gentle AP30 Transfection Reagent is not recommended to vortex vigorously, and it is recommended to use a pipette to mix by gentle and repeated pipetting.

- **Other common questions.**
  ① The centrifugation protocols described in this manual are standardized at room temperature, 300g, for 5 minutes.
  ② After transfection, add 1 mL of complete medium and mix gently to terminate the transfection process and mitigate the potential impact of the transfection reagent on subsequent cell centrifugation (Note: The slight viscosity of the transfection reagent is typical and does not indicate an issue);
  ③ After centrifugation, the cell pellet may not be readily visible, especially when working with low cell numbers. This is a common occurrence and does not necessarily indicate a procedural issue.

This product is exclusively intended for professional scientific research purposes and is not suitable for clinical diagnosis, treatment, or any application in food or pharmaceutical products.

In the event of technical challenges or issues encountered during experimentation, users may seek technical support via the email provided below. We sincerely welcome constructive feedback and valuable suggestions regarding this reagent to enhance its performance and utility!

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References

【1】Sun, Y., Wu, X., Li, J. *et al.* Phase-separating peptide coacervates with programmable material properties for universal intracellular delivery of macromolecules. *Nat Commun* 15, 10094 (2024).

【2】Sun, Y., Lau, S.Y., Lim, Z.W. *et al.* Phase-separating peptides for direct cytosolic delivery and redox-activated release of macromolecular therapeutics. *Nat. Chem.* 14, 274–283 (2022).