A&A BIOTECHNOLOGY
innovating life science

Manual

GeneJect™

DNA, siRNA / miRNA transfection reagent.

cat #	size
4100-01	100 µl
4100-05	500 µl
4100-15	1.5 ml

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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Advantages

- Suitable for DNA, siRNA and miRNA transfection
- Low cell toxicity
- Efficient transfection of wide variety of primary cells and cell lines

Description

GeneJect™ is a transfection reagent, which is suitable for the delivery of plasmid DNA and short oligonucleotides, such as siRNAs as well as miRNA mimics and inhibitors.

GeneJect™ is less toxic when compared with lipofection. Toxicity is higher at low cell confluency.

GeneJect™ is efficient in the transfection of common cell lines of different origin, including HeLa, HEK293, PPC-1, HaCaT, A549, BEAS-2B, CCD19Lu, THP-1 and KOP (fetal bovine oropharynx cells). It also enables transfection of low level transfection cells including MDBK (Madin Darby Bovine Kidney cells).

GeneJect™ is efficient in the transfection of wide variety of primary cells, such as human primary keratinocytes, primary skin fibroblasts, melanocytes and monocytes.

100 µl of GeneJect™ allows for up to 50 transfections in 12-well tissue culture plates format.

Contents

	4100-01	4100-05	4100-15	storage
GeneJect™ - transfection reagent	100 µl	500 µl	1.5 ml	4 °C

Additional equipment and reagents

- Opti-MEM™, Reduced Serum Medium (Thermo Fisher Scientific, cat# 31985062) or equivalent.

Comments

- As the transfection with GeneJect™ is relatively fast, the growth media containing GeneJect™ / plasmid DNA, siRNA, miRNA transfection complexes can be removed and replaced with normal growth media after 6 hour or next day.
- 50 - 70% confluency corresponds to $5x10^4$ human primary keratinocytes per one well of 12-well plate.
- Similarly to other transfection reagents, GeneJect™ may slightly inhibit cell proliferation.

- GeneJect™ can be stored at 4 °C for at least 6 months. For 12-month storage, GeneJect™ can be aliquoted and freezed at -20 °C. However, multiple freeze-thaw cycles should be strictly avoided.

Plasmid DNA transfection

Good practices for effective plasmid DNA transfection

- Store appropriately GeneJect™ (4 °C) and plasmid DNA (4 °C)
- Ensure that cells have been passaged more than twice and less than 20 times prior to transfection.
- Discard over confluent cells
- Regularly check for mycoplasma contaminations
- Use a reporter gene to control and optimize transfection conditions
- Serum quality may drastically affect transfection efficiency. Check each new batch of serum or trypsin versus cell viability as well as transfection efficiency

Plasmid DNA transfection protocol

These instructions are for transfecting cells in 12-well tissue culture plates with surface area of 4 cm^2. For transfections in tissue culture plates with a surface area other than 4 cm^2, please adapt amounts according to the **Table 1**.

Day 1

1. Seed cells into a 12-well tissue culture plate at **50 - 70% confluency** in **1000 µl** normal growth medium per well.

Day 2

1. Mix **400 ng** of **plasmid DNA** in **100 µl** of **Opti-MEM™** and vortex briefly.
2. Add **2 µl** of **GeneJect™** and vortex briefly.
3. Incubate for **30 minutes** at **room temperature**.
4. Add **900 µl** of **normal growth media** and vortex briefly.
5. Remove old growth media from the cells.
6. Add new growth media containing GeneJect™ / plasmid DNA transfection complexes to the cells.
7. Incubate for **24 - 72 hours** at temperature suitable for Your cell type.

Day 3 - 5

1. Investigate gene of interest expression.

Table 1. Plasmid DNA transfection guidelines according to the cell culture vessel per well

The amounts are optimized for human primary keratinocytes. Increasing the amount of plasmid DNA proportionally with the amount of GeneJect™ may enhance transfection efficiency in some cell lines.

Please note the given amounts and ratio of reagents are only a starting point general guidelines for most successful transfection depending on nucleic acid type, size and cell type. For optimisation please review the **Optimisation guidelines for plasmid DNA**.

plate	Plasmid DNA (ng)	GeneJect™ (µl)	Opti-MEM™ (µl)	Normal growth media (µl)
24-well	200	1	50	400
12-well	400	2	100	900
6-well	800	4	200	1800
⌀ 10 cm	4000	10	1000	9000

Optimisation guidelines for plasmid DNA

- Test different plasmid DNA amounts within range of 0.5x to 1.5x compared to the values given in **Table 1.**
- Test different GeneJect™ amounts within range of 1x to 3x compared to the values given in **Table 1.**

Tips to improve cell viability of sensitive cells

- Replace medium after 4 h
- Decrease DNA amount to 0.5x
- Analyze transfection at shorter time course point (i.e. 24 h after transfection versus 48 h)
- Check that the target gene does not affect cell viability

siRNA / miRNA transfection

Good Practices for effective siRNA / miRNA transfection

- Store appropriately GeneJect™ (4 °C) and RNA (-20 °C)
- Ensure that cells have been passaged more than twice and less than 20 times prior to transfection.
- Discard over confluent cells

- Regularly check for mycoplasma contaminations
- Use a positive control (e.g. housekeeping gene namely GAPDH, HPRT or fluorescently labeled siRNA)
- Use negative control (e.g. mismatch or non-targeting sequence)
- Check the half-life of the targeted mRNA and protein product of interest. Measure the silencing effect in a time course mode at 24 and 48 and 72 and 96 h from the transfection
- Serum quality may drastically affect transfection efficiency. Check each new batch of serum or trypsin versus cell viability as well as transfection efficiency

siRNA / miRNA transfection protocol

These instructions are for transfecting cells in 12-well tissue culture plates with surface area of 4 cm^2. For transfections in tissue culture plates with a surface area other than 4 cm^2, please adapt amounts according to the **Table 2.**

Day 1

1. Seed cells into a 12-well tissue culture plate at **50 - 70% confluency** in **1000 µl** normal growth medium per well.

Day 2

1. Mix **30 pmol** of **siRNA / miRNA** in **100 µl** of **Opti-MEM™** and vortex briefly.
2. Add **2 µl** of **GeneJect™** and vortex briefly.
3. Incubate for **30 minutes** at **room temperature**.
4. Add **900 µl** of **normal growth media** and vortex briefly.
5. Remove old growth media from the cells.
6. Add new growth media containing GeneJect™ / siRNA / miRNA transfection complexes to the cells.
7. Incubate for 24 - 72 hours at temperature suitable for Your cell type.

Day 3 - 5

1. Investigate gene of interest expression.

Table 2. siRNA / miRNA transfection guidelines according to the cell culture vessel per well

The amounts are optimized for human primary keratinocytes. Increasing the amount of siRNA / miRNA proportionally with the amount of GeneJect™ may enhance transfection efficiency in some cell lines.

Please note the given amounts and ratio of reagents are only a starting point general guidelines for most successful transfection depending on nucleic acid type, size and cell type. For optimisation please review the **Optimisation guidelines for siRNA / miRNA**.

plate	siRNA / miRNA (pmol)	GeneJect™ (µl)	Opti-MEM™ (µl)	Normal growth media (µl)
24-well	15	1	50	400
12-well	30	2	100	900
6-well	60	4	200	1800
⌀ 10 cm	300	10	1000	9000

Optimisation guidelines for siRNA / miRNA

- Test different siRNA / miRNA concentration ranging from 10 to 50 nM (working concentration)
- Increasing the ratio of GeneJect™ to siRNA / miRNA may further increase the efficiency of transfection. Test the GeneJect™ to siRNA / miRNA ratio from initial 1 µl / 15 pmol to 1.5 µl / 15 pmol.
- Use 50% confluence cells in the transfection

Tips to improve cell viability of sensitive cells

- Replace medium after 4 h
- Analyze transfection at shorter time course point (i.e. 24 h after transfection versus 48 h)
- Check that the silencing the target gene does not affect cell viability

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