NATE™

Nucleic acid transfection & transduction enhancer

Catalog code: lyec-nate

www.invivogen.com/nate-transfection-transduction-enhancer

For research use only

Version 24H01-AK

PRODUCT INFORMATION

Contents

• 2 vials of NATE™(approximately for 100 reactions); provided in evaporated form

Storage and stability

- NATE™ is provided as a translucent film and shipped at room temperature. Upon receipt, store product at -20 °C.
- Upon resuspension of NATE™ prepare aliquots and store resuspended product at -20 °C. Resuspended product is stable for 6 months when properly stored.
- Avoid repeated freeze-thaw cycles.

Quality control

- Purity: >95% UHPLC
- Absence of bacterial contamination (i.e. lipoproteins and endotoxin) has been confirmed using HEK-Blue™ hTLR2 and hTLR4 cellular assays, respectively.
- Biological activity has been confirmed using functional assays.

BACKGROUND

The principle obstacle for 'foreign' nucleic acids (such as plasmids) during eukaryotic cell transfection is their own detection by cytosolic DNA and RNA sensors such as RIG-I Like receptors (RLRs), cyclic GMP-AMP synthase (cGAS), and the inflammasome Absent in melanoma 2 (AIM2)[1]. Additionally, they need to evade other defensive cellular strategies such as autophagy[2]. The activation of 'foreign nucleic acid' sensing signaling cascades, frequently leads to low transfection efficiency and reduced cell viability.

METHODS

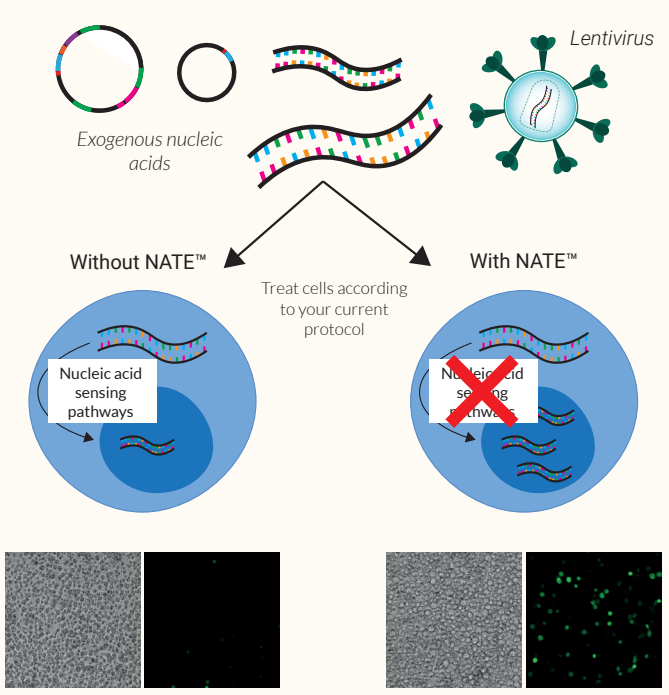
Preparation of stock suspension (100 x NATE™)

- Add 250 µl of DMSO to the evaporated product in the vial.
- Vortex vigorously to ensure the film is completely dissolved.
- Add 250 µl of sterile endotoxin-free water and vortex again.
- Prepare aliquots and store at -20 °C.

On the next page (below) are the validated protocols for using NATE™ in both transient/stable transfections and lentiviral transduction. They were tested in THP-1, RAW 264.7, and primary T cells.

PRODUCT DESCRIPTION

NATE™, a non-toxic, highly efficient *nucleic acid transfection and transduction enhancer*, has been designed specifically to increase transfection and lentiviral transduction efficiencies in hard-to-transfect cell lines, such as THP-1, RAW 264.7, and primary T cells. NATE™ is a propriatary blend of innate immune system inhibitors that target the host's defensive strategies, thereby protecting the 'foreign' nucleic acids and improving the gene transfer success. NATE™ is simply added 30 minutes before all commonly used protocols. It is adaptable for both transient and stable transfections including various transfection reagents and electroporation. It can be used for lentiviral transduction using static or spinoculation methods. Notably, NATE™ is gentle on cells and does not induce any further toxicity into the cell culture.



Transient and stable transfection with NATE™

1. Patrick, K.L. *et al.* 2016. For Better or Worse: Cytosolic DNA Sensing during Intracellular Bacterial Infection Induces Potent Innate Immune Responses. J Mol Biol 428, 3372-3386. 2. Gui, X. *et al.* 2019. Autophagy induction via STING trafficking is a primordial function of the cGAS pathway. Nature 567, 262-266. 3. Brielmeier, M. *et al.*, 1998. Improving stable transfection efficiency: antioxidants dramatically improve the outgrowth of clones under dominant marker selection. Nucleic Acid Res. 26(9):2082-5. 4. Liu. L. *et al.*, 2011. Transfection optimization for Primary Human CD8+ cells. J Immunol Methods. 372(1-2):22-29.

TECHNICAL SUPPORT
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TRANSFECTION PROTOCOL

Below is a detailed protocol for using NATE™ in both transient and stable transfection of THP-1 and RAW 264.7 cells. This protocol can be adapted and used for a number of variations of both transent and stable transfection, including different cell culture plate sizes, varying transfection methods, and a range of plasmid sizes.

a) Cell preparation

1. Prepare the hard to transfect cells in complete culture medium as usual.

- *Note for RAW 264.7 transfection: The cells must be seeded 24 hours before starting the transfection method.*
- *Note for THP-1 transfection: The efficiency of transfection depends on how the cells are cultivated. Subdivide the cells every 3 days to a starting density of 0.5 x 10^6 cells/ml.*
- *Note for stable THP-1 transfection: A rich culture medium is recommended for use during transfection, in which the normal culture medium is supplemented with 20% serum, 20% conditioned medium, sodium pyruvate, non-essential amino acids, and anti-oxidant agents as described previously[3].*

b) Addition of NATE™

2. Add 100X stock solution of NATE™ to a final concentration of 1X. The amount added (**1% addition**) will vary depending on the plate used and the volume of cell culture medium *(see table below)*.
3. Swirl to distribute uniformly throughout the cell population.
4. Incubate for a minimum 30 minutes under normal cell culture conditions.

c) Transfection

5. Perform gene transfer method (including polymer or lipid-based transfection, electroporation) as per the manufacturer's instructions.
Note: If the gene transfer method requires the medium to be removed shortly after transfection, NATE™ must be added again to the new medium for the additional incubation time.

6a. ***For transient transfection*** - incubate under appropriate conditions for 24-48 hours to allow for gene expression before assaying.

6b. ***For stable transfection*** - apply antibiotic selection 2-4 days after the transfection. Delaying the selection allows the cells to fully recover from the transfection.
Note for stable THP-1 transfection: When the selection efficiency begins to increase,we recommend you remove the dead cells with an appropriate commerically available kit as described previously[4].

LENTIVIRAL TRANSDUCTION PROTOCOL

Below is a detailed protocol for using NATE™ for lentiviral transduction of primary CD4+ T cells. This protocol can be adapted and used for a number of variations, including different cell types, culture plate sizes, and varying transduction methods (static or spinoculation).

a) Cell preparation

1. Isolate and expand your T cells as usual.
Change the medium one day prior to adding NATE™. Add the cytokine cocktail according to your T cell expansion protocol (e.g. IL-2).

b) Addition of NATE™

2. Add 100X stock solution of NATE™ to a final concentration of 1X. The amount added (**1% addition**) will vary depending on the plate used and the volume of cell culture medium *(see table below)*.
3. Swirl to distribute uniformly throughout the cell population.
4. Incubate for a minimum 30 minutes under normal cell culture conditions.

c) Lentiviral transduction

5. Perform gene transfer method (static or spinoculation) as per the manufacturer's instructions.
Note: If the gene transfer method requires the medium to be removed shortly after transfection, NATE™ must be added again to the new medium for the additional incubation time.

6. Incubate under appropriate conditions for 24-48 hours to allow for gene expression before assaying.

RELATED PRODUCTS

Product	Description	Cat. Code
Recombinant human IL-2	CHO-derived cytokine	rcyc-hil2
Blasticidin	Selective antibiotic	ant-bl-1
G418 (Geneticin)	Selective antibiotic	ant-gn-1
Hygromycin B Gold	Selective antibiotic	ant-hg-1
Puromycin	Selective antibiotic	ant-pr-1
Zeocin®	Selective antibiotic	ant-zn-1

Set-up examples with NATE™

Below are examples of the experimental set-up for transfection or transduction of either RAW 264.7, THP-1, or T cells with NATE™.

	FOR TRANSFECTION		FOR TRANSDUCTION		
	RAW 264.7	THP-1	THP-1	CD4+ T cells	
Plate size	24-well	12-well	48-well	96-well	48-well
Culture volume	1 ml	1 ml	1 ml	0.2 ml	1 ml
Seeding density (cells/well)	2 x 10^5	5 x 10^5	2.5 x 10^5	2 x 10^5	1 x 10^6
Transfection reagent used	Lipofectamine® LTX	Gene*XPlus*	-	-	-
Volume of 100x NATE™	10 µl	10 µl	10 µl	2 µl	10 µl

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