NATE™

Nucleic acid transfection enhancer

Catalog code: lyec-nate

https://www.invivogen.com/nate

For research use only

Version 19D29-ED

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

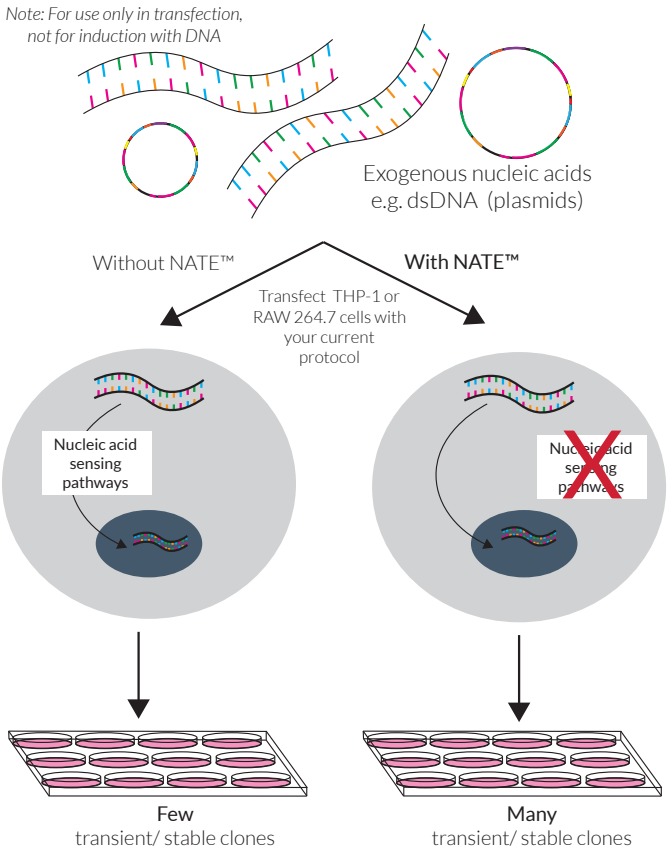
- Purity: >95% UHPLC
- Absence of bacterial contamination (i.e. lipoproteins and endotoxin) has been confirmed using HEK-Blue™ hTLR2 and hTLR4 cellular assays, respectively.
- Biological activity has been confirmed using transfection assays.

PRODUCT DESCRIPTION

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[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.

NATE™, a *nucleic acid transfection enhancer*, has been designed specifically to increase transfection efficiency in hard-to-transfect cell lines such as THP-1 and RAW 264.7. NATE™ is a propriatary blend of innate immune system inhibitors that targt the host's defensive strategies and thereby protects the plasmid during transfection. NATE™ is simply added 30 minutes before all commonly used protocols for both transient and stable transfections including various transfection reagents and electroporation. Specifically for RAW 264.7 cells, stable clones can be obtained within 2 weeks without the need to grow pools of clones before the individual clones are selected. Notably, NATE™ is gentle on cells and does not induce any further toxicity into the cell culture.

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.



Transient and stable transfection with NATE™

METHODS

Below (and on the next page) are the steps for preparing a single vial of NATE™ for use as well as a validated protocol for using NATE™ in both transient and stable transfections.

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.

TECHNICAL SUPPORT
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Using NATE™ in both transient and stable transfection

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 (6-, 12-, and 24-well plates), varying transfection methods, and a range of plasmid sizes.

a) Cell preparation

1. Prepare the hard to transfect cells (i.e. THP-1 or RAW 264.7 cell lines) in complete culture medium as usual.

- *Note for RAW 264.7 transfection: The cells must be seeded 24 hours prior to adding NATE™ and starting the transfection method.*
- *Note for THP1 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 THP1 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 pyurvate, 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.
Note: For example, when using 1 mL of cell culture in the transfection add 10 µL of NATE™ to your cells.

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, viral transduction or 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 THP1 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].

Example transfection set-up with NATE™

Below in the table is an example of the experimental set-up for transfection of either THP-1 or RAW 264.7 cells with NATE™.

	THP-1	RAW 264.7
Plate size	12-well	24-well
Culture Volume	1 ml	1 ml
Seeding Cell Density	5 x 10^5 c/w/ml	2 x 10^5 c/w/ml
Transfection reagent used	Gene*XPlus*	Lipofectamine® LTX
Volume of 100x NATE™	10 µl	10 µl

RELATED PRODUCTS

Product	Description	Cat. Code
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