USER GUIDE

NANOBLOCK

Delivery Systems

NANOBLOCK Nucleic Acid Delivery Kit

WWW.NESBIOTECH.COM

NES BIOTECHNOLOGY

Table of Contents

NANOBLOCK DELIVERY SYSTEM

Introduction

The efficient delivery of bioactive molecules into mammalian cells is a central aspect of research involving cell biology and medical applications. NANOBLOCK Delivery System, gold nanoparticles (AuNP) functionalized to a universal cargo DNA, can be a convenient and efficient method of delivering nucleic acids, proteins, and antibodies to a wide variety of cells including primary cells and animals. In addition, this system can be achieved without causing significant cytotoxicity, even during long incubation periods (1– 5 days). Such properties are advantageous in biological applications of bioactive molecules delivery systems, which require careful safety measures.

Overview – The Principle of NANOBLOCK Delivery Systems

Applications

NANOBLOCK delivery system is easily applicable to the delivery of various bioactive molecules into numerous cells in vitro and in vivo without cytotoxicity. This lego-like AuNP delivery system can be used universally for biological experiments including gene expression regulation, intracellular signaling, and transcriptional regulation.

NANOBLOCK Delivery System	Application	Conjugated Bioactive Molecule
NANOBLOCK-Nucleic Acid Delivery Kit	Transient gene knockdown	(ASO) : p53
		(shRNA) : p53, MCL1
	Alternative splicing modulation	(ASO) : MCL1, BCL6
	Sequestering of transcription factor	(DSO) : Estrogen response elements, p53 response elements
	Inhibition of miRNA activity	(AMO) : miR-29b, miR-21
	Inhibition of protein activity	(Aptamer) : beta-catenin, p50
	Synthesis of protein	(mRNA): BAX, dsRED, GFP
NANOBLOCK-Proteins Delivery Kit	Cancer research - intercellular signaling - cell cycle regulation - apoptosis - oncogenesis - transcriptional regulation	(Protein) : BIM, FOXL2, BCL-xL, EGF, RraAV1, Rnase III, AcrA, Enolase (Peptide) : TM-JM1/2, Lamin 406-567, Lamin 406-665
	Antimicrobial research	(AMP) : A3-APO, HPN3, HPA3P

ASO : antisense oligonucleotide
shRNA : small hairpin RNA
DSO : double-stranded oligonucleotide
AMO : anti-miRNA oligonucleotide
mRNA : messenger RNA
AMP : anti-microbial peptide

Example of bioactive molecules successfully applied to biological experiments with NANOBLOCK Delivery Systems

Cell Line	Cell Type	Culture Property	Species
HeLa	Cervical epithelial adenocarcinoma	Adherent	Human
293T	Embryonic kidney (epithelial)	Adherent	Human
K562	Chronic Myelogenous Leukemia (Lymphoblast-like)	Suspension	Human
LoVo	Colon epithelial adenocarcinoma	Adherent	Human
MCF-7	Breast epithelial adenocarcinoma (Mammary gland)	Adherent	Human
KGN	Ovarian granulosa cell (Solid Carcinomas)	Adherent	Human
HepG2	Hepatoblastoma (epithelial-like morphology)	Adherent	Human
A549	Lung epithelial adenocarcinoma	Adherent	Human
A431	Skin/epidermis (epithelial)	Adherent	Human
H1299	Lung epithelial carcinoma	Adherent	Human
COV434	Ovary (Polygonal & fusiform)	Adherent	Human
J1 mouse embryonic stem cell		Adherent	Mouse
Primary cells			
Cervical squamous carcinoma primary cell		Adherent	Human
primary granulosa cell		Adherent	Rat
Animals			
FvB mouse			
Sprague–Dawley rat			
BALB/c nu/nu immunodeficient mouse (Xenograft tumor model, LoVo cells)			
BALB/c nu/nu immunodeficient mouse (Xenograft tumor model, HeLa cells)			

Example of cells efficiently transported bioactive molecules with NANOBLOCK Delivery Systems

RELATED PRODUCTS

High performance transfection efficiency of proteins into the living cells

Catalog No.	Product	Quantity
NES001-01	NANOBLOCK Protein Delivery Kit	1 ml
NES001-02	NANOBLOCK Protein Delivery Kit Bulk	5 X 1 ml

Delivery of non-histag proteins are required additional customized aptamer construction. If you would like to order the customized products, please contact our Technical Support Department by email, info@nesbiotech.com.

High performance transfection efficiency of antibodies into the living cells

Catalog No.	Product	Quantity
NES002-01	NANOBLOCK Antibody Delivery Kit	1 ml
NES002-02	NANOBLOCK Antibody Delivery Kit Bulk	5 X 1 ml

High performance transfection efficiency of nucleic acids into the living cells

Catalog No.	Product	Quantity
NES003-01	NANOBLOCK Nucleic Acid Delivery Kit	1 ml
NES003-02	NANOBLOCK Nucleic Acid Delivery Kit Bulk	5 X 1 ml

CONTACT US

Orders

+82-2-820-5241
+82-2-822-5241
info@nesbiotech.com
www.nesbiotech.com

Technical Support

If you need assistance with your experiments using this product, please contact our Technical Support Department: **info@nesbiotech.com**

NANOBLOCK
NUCLEIC ACID DELIVERY KIT

The Nanoblock-Nucleic Acid Delivery Kit is a unique gold nanoparticle (AuNP)-based nuleic acid delivery system that can be used to deliver biologically active DNA or RNA into living cells.

The Nanoblock-Nucleic Acid Kit is a simple, efficient, and versatile nucleic acid delivery system that allows any poly-adenylated DNA or RNA to be loaded without additional modifications and delivered into mammalian living systems in a manner independent of their size. And this system could deliver various nucleic acids into variety of cell types (on primary and stem cells, indicating that its use is not limited to fast-dividing cells) in vitro without showing cytotoxicity. Furthermore, this system was also effective for the local and systemic targeted delivery of nucleic acids in vivo. Therefore, the Nanoblock-Nucleic Acid Delivery Kit may be used in applications relevant to intercellular signaling, cell cycle regulation, apoptosis, oncogenesis and transcriptional regulation.

KIT CONTENTS

Nanoblock-Nucleic Acid Delivery Kit (NES003-01)

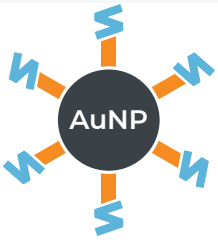
Catalog No.	Reagent	Quantity	Storage/Stability
NES003-01-01	$AuNP^{dT}$ Reagent (25nM)	1 ml	4°C for 2 month
NES003-01-02	Control mRNA	20 µl	4°C for 2 month
NES003-01-03	5x NA Binding buffer	1 ml	RT for 1 year
NES003-01-04	NA salt buffer	1 ml	RT for 1 year

*Additional Materials Required.

Sterile 1.5 ml microcentrifuge tube

PROTOCOL

QUICK GUIDE

Process		Description	Materials	96-well	24-well	6-well
1		**The cells** - Seed to be 70 ~ 80% confluent	Number of the cells ($x10^4$)	1.5 ~ 0.5	10 ~ 5	50 ~ 25
2		**Nucleic acid solution** - Prepare nucleic acid solution with nuclease free water - Incubate at 80 °C for 10 mins and chill on the ice for 10 mins	Nucleic acid solution (µl)	4	20	80
3		 **AuNP^{dT} Reagent** Add AuNP^{dT} reagent, 5X NA binding buffer into nucleic acid solution, and gently tapping.	AuNP^{dT} Reagent (µl)	2	10	40
			5X NA binding buffer (µl)	2	10	40
			Nuclease free water (or NA salt buffer)	2	10	40
4		 **Conjugation** **[AuNP^{dT} - poly-adeylated Nucleic acid]** - Incubate for 10 mins at 55 °C and then chill on the ice for 30 mins				
5		**Transfection** - Add conjugated solution to the cells	Conjugated solution (µl)	10	50	200

DETAILED INSTRUCTION

Step 1. Cells Preparation

Protocol

1) The day before nucleic acid delivery experiment, seed/split the cells at appropriate density on appropriate culture vessel for your experiment. We suggest cell number to seed in table 1.

2) The suitable cell density will depend on the growth rate and the condition of the cells. Cells should not be more than 70-80% confluent at the time of experiment.

Experimental consideration

Table 1: Suggested number of cells to seed.

Culture vessel	Number of adherent cells	Number of suspension cells	Cell overlay volume
96 well	0.5 – 1.5 X 10^4	0.5 – 1 X 10^5	90 µL
24 well	0.5 – 1 X 10^5	0.5 – 5 X 10^5	450 µL
6 well	2.5 – 5 X 10^5	5 – 20 X 10^5	1.8 mL

Step 2. AuNP^{dT} / Nucleic acid Conjugation

Protocol

1) Prepare nucleic acid soltuion in microtube.

- Mix the nucleic acids and nuclease free water
- Incubate the nucleic acid solution at 80 °C for 10 mins and then chill on the ice for 10 mins

[Note] Low concentration of nucleic acids can lead to AuNP aggregation. We recommend to add at least 50 nucleic acids to 1 AuNP in molar ratio.

2) Construct conjugates of AuNP^{dT} and poly-adeylated nucleic acid.

- Add AuNP^{dT} reagent into nucleic acid solution and gently tapping.
- Add 5X NA binding buffer into AuNP^{dT}-nucleic acid mixture and gently tapping.
- Incubate at 55°C for 10 mins and then chill on the ice for 30 mins.

** The amounts of reagents depending on culture vessel are in table 2.*

Experimental consideration

Table 2: Suggested amount of nucleic acid and AuNP$_{dT}$

Culture vessel	Nucleic acid solution	5X NA Binding buffer	Nuclease free water (or NA salt buffer)	AuNP^{dT}	Total Volume
96 well	4 µL	2 µL	2 µL	2 µL	10 µL
24 well	20 µL	10 µL	10 µL	10 µL	50 µL
6 well	80 µL	40 µL	40 µL	40 µL	200 µL

** Because nucleic acids differ one from another, reflecting a variety of physical properties, conjugation efficiency with AuNP reagent are variable. NA salt buffer can increase conjugation efficiency of AuNP and nucleic acids, but high concentration of NA salt buffer can lead AuNP aggregation. It is recommended that NA salt buffer does not exceed 10% of the reaction mixture. So, users need to determine optimum conditions to deliver your nucleic acids.*

[Note] The presence of NaCl (>300 mM) or $MgCl_2$ (>10 mM) can precipitate the AuNPs. If NaCl and $MgCl_2$ are present as high concentration in your nucleic acids sample, we recommend removing it before proceeding with the delivery assay.

** Any impurities, contaminants present with your sample might affect the delivery efficiency. Also, additives such as detergents, glycerol, sodium azide may inhibit the delivery. Consequently, we suggest using nucleic acids as pure as possible.*

Step 3. Transfection

Protocol

1) Disperse onto the cells growing in their regular culture medium (with serum).

2) Incubate the cells at 37 °C in a CO_2 incubator under standard conditions until the evaluation of the nucleic acid delivery efficiency (1-48 h). Incubation time will depend on your experimental purpose (for example, incubate 1 ~4 h to detect delivered nucleic acids, and incubate over 24 h to determine cell toxicity of delivered nucleic acids).

Experimental consideration

** Some nucleic acids can degrade in media containing serum (ex – long mRNA). AuNP reagent can be used onto cells in serum-free media. In this case, replace the complete culture medium by serum-free medium. After 3-4 h, add some serum-containing medium if further incubation time is necessary. This procedure can be more efficient to deliver certain nucleic acids.*

Appendix

1 QUALITY CONTROLS

2 TROUBLESHOOTING

1. Low delivery efficiency

Check point	Suggestion
Nucleic acid purity	Make sure that the your nucleic acid is highly pure and devoid of additives such as NaCl, $MgCl_2$ BSA or detergents.
$AuNP^{dT}$ reagent amount	Optimize the quantity of $AuNP^{dT}$ reagent as described in the table 2.
Nucleic acid amount	Optimize the $AuNP^{dT}$ / nucleic acid ratio. We recommend using at least 50 times as much nucleic acid as gold.
Cell density	A non-optimal cell density at the time of nucleic acid delivery can lead to insufficient uptake. The optimal confluence should range from 70 to 80%.
Cell condition	Cells that have been in culture for a long time (> 8 weeks) may become resistant to the delivery. Use freshly thawed cells that have been passaged at least once. Cells should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) alters considerably the delivery efficiency.
Cell culture medium composition	For some cells, delivery efficiency can be increased without serum or under reduced serum condition. Thus, assay these cells in serum-free medium during the first 4 h of incubation.
Incubation time	The optimal time range between delivery and assay varies with cells, type of nucleic acid, kinetics of biological function, etc. The delivery efficiency can be monitored after 4 to 96h. Control mRNA can be used to quantitatively monitored delivery kinetics.
Old $AuNP^{dT}$ / Nucleic acid complexes	$AuNP^{dT}$ / Nucleic acid complexes must be freshly prepared every time. Complexes prepared and stored for more than 1 hour can be aggregated. Depending on the nucleic acid, reduce this time to avoid the aggregation which may occur during the complex formation.
Positive control	Ensure that your experiment is properly set up and includes a positive control. The Control mRNA provided in the kit can be used as positive control for delivery efficiency.
$AuNP^{dT}$ reagent storage	Delivery efficiency can slowly decrease if $AuNP^{dT}$ reagent is kept more than one week at room temperature.

2. Cellular toxicity

Check point	Suggestion
Concentration of AuNP^{dT} / Nucleic acid too high	Decrease the amount of AuNP^{dT} / Nucleic acid complexes added to the cells by lowering the Nucleic acid amount or the AuNP^{dT} reagent. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously.
Unhealthy cells	Check cells for contamination Use new batch of cells Ensure culture medium condition (pH, type of medium used, contamination etc) Cells are too confluent or cell density is too low Verify equipments and materials
Nucleic acid is cytotoxic	Use suitable controls such as cells alone, AuNP^{dT} reagent alone or mock delivery.
Incubation time	Reduce the incubation time of complexes with the cells. Delivery medium can be replaced by fresh medium after 3 to 24 h if necessary.
Nucleic acid quality	Use high quality nucleic acid as impurities could lead to cell death.

3 EXAMPLE PROTOCOL AND RESULT

Delivery of control mRNA using a 24-well plate

1. Seed 0.5 to 1 × 10^5 cells per well in a 24-well plate or on a cover slip. Let grow overnight.
2. Mix 5 µl of control mRNA and 15 µl of nuclease free water.
3. Incubate at 80°C for 10 mins and then chill on the ice for 10 mins.
4. Add 10 µl of AuNP reagent into nucleic acids solution and gently tapping.
5. Add 10 µl of 5X NA binding buffer, 10 µl of nuclease free water into AuNP-nucleic acid mixture and gently tapping.
6. Incubate at 55 °C for 10 mins and then chill on the ice for 30 mins.
7. Disperse mixture 6 onto cells in serum-free media.
8. Incubate cells at 37°C in a CO_2 incubator.
9. After 4 h, add same volume of serum-containing media.
10. Incubate for additional 20 h.
11. Observe delivered nucleic acids by fluorescent microscopy.

4 REFERENCE

1. Kim, J.-H. et al. A functionalized gold nanoparticles-assisted universal carrier for antisense DNA. Chemical Communications 46, 4151-4153, doi:10.1039/C0CC00103A (2010).
2. Ryou, S. M. et al. Delivery of shRNA using gold nanoparticle-DNA oligonucleotide conjugates as a universal carrier. Biochemical and biophysical research communications 398, 542-546, doi:10.1016/j.bbrc.2010.06.115 (2010).
3. Kim, D. W. et al. Modulation of biological processes in the nucleus by delivery of DNA oligonucleotides conjugated with gold nanoparticles. Biomaterials 32, 2593-2604, doi:10.1016/j.biomaterials.2010.11.074 (2011).
4. Kim, J. H. et al. Effective delivery of anti-miRNA DNA oligonucleotides by functionalized gold nanoparticles. Journal of biotechnology 155, 287-292, doi:10.1016/j.jbiotec.2011.07.014 (2011).
5. Ryou, S.-M. et al. Inhibition of xenograft tumor growth in mice by gold nanoparticle-assisted delivery of short hairpin RNAs against Mcl-1L. Journal of biotechnology 156, 89-94, doi:https://doi.org/10.1016/j.jbiotec.2011.07.037 (2011).
6. Ryou, S. M. et al. Gold nanoparticle-assisted delivery of small, highly structured RNA into the nuclei of human cells. Biochemical and biophysical research communications 416, 178-183, doi:10.1016/j.bbrc.2011.11.020 (2011).
7. Kim, Y. J. et al. Enhanced protein-mediated binding between oligonucleotide–gold nanoparticle composites and cell surfaces: co-transport of proteins and composites. Journal of Materials Chemistry 22, 25036-25041, doi:10.1039/C2JM34047J (2012).
8. Yeom, J.-H. et al. Inhibition of xenograft tumor growth by gold nanoparticle-DNA oligonucleotide conjugates-assisted delivery of BAX mRNA. PLOS ONE 8, e75369, doi:10.1371/journal.pone.0075369 (2013).
9. Ryou, S. M. et al. Gold nanoparticle-DNA aptamer composites as a universal carrier for in vivo delivery of biologically functional proteins. Journal of controlled release : official journal of the Controlled Release Society 196, 287-294, doi:10.1016/j.jconrel.2014.10.021 (2014).
10. Kim, D. et al. Inhibition of discoidin domain receptor 2-mediated lung cancer cells progression by gold nanoparticle-aptamer-assisted delivery of peptides containing transmembrane-juxtamembrane 1/2 domain. Biochemical and biophysical research communications 464, 392-395, doi:10.1016/j.bbrc.2015.06.044 (2015).
11. Yeom, J.-H. et al. Gold nanoparticle-DNA aptamer conjugate-assisted delivery of antimicrobial peptide effectively eliminates intracellular Salmonella enterica serovar Typhimurium. Biomaterials 104, 43-51, doi:https://doi.org/10.1016/j.biomaterials.2016.07.009 (2016).
12. Yeom, J.-H. et al. Intracellular delivery of recombinant proteins via gold nanoparticle–DNA aptamer composites is independent of the protein physicochemical properties and cell type. Journal of Industrial and Engineering Chemistry 45, 5-10, doi:https://doi.org/10.1016/j.jiec.2016.09.043 (2017).
13. Lee, B. et al. Antimicrobial peptide-loaded gold nanoparticle-DNA aptamer conjugates as highly effective antibacterial therapeutics against Vibrio vulnificus. Scientific Reports 7, 13572, doi:10.1038/s41598-017-14127-z (2017).

THANK YOU FOR CHOOSING

NANOBLOCK

Delivery Systems

NES
BIOTECHNOLOGY

+82-2-820-5241
+82-2-822-5241
info@nesbiotech.com
www.nesbiotech.com