USER GUIDE

NANOBLOCK

Delivery Systems

NANOBLOCK Antibody Delivery Kit

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NES BIOTECHNOLOGY

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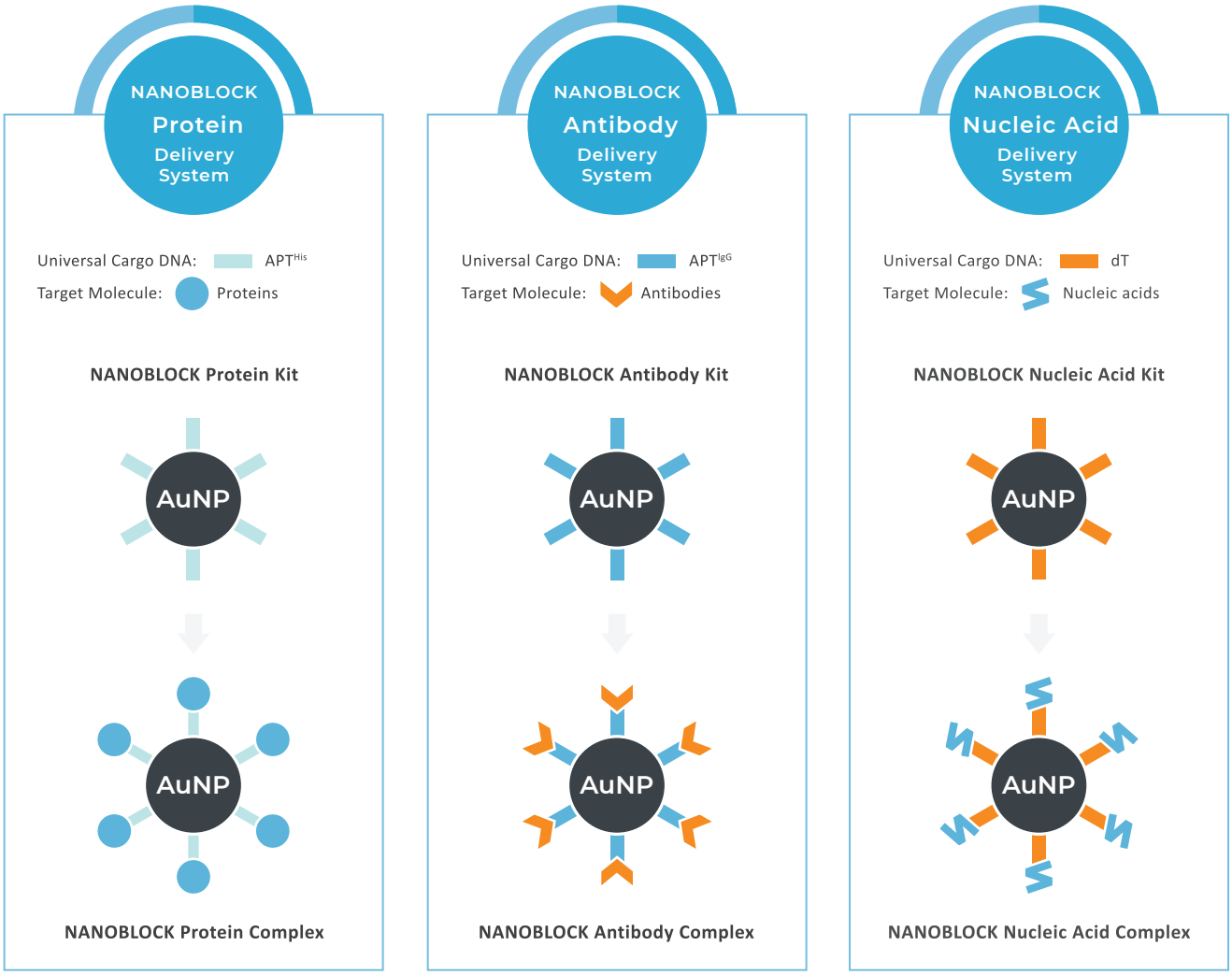
NANOBLOCK DELIVERY SYSTEM

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

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Orders

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Technical Support

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NANOBLOCK
ANTIBODY DELIVERY KIT

The Nanoblock -Antibody Delivery Kit is a unique gold nanoparticle (AuNP)-based antibody delivery system that can be used to deliver biologically active antibodies into living cells.

The Nanoblock-Antibody Delivery Kit is a simple, efficient, and versatile antibody delivery system that allows any antibodies containing Fc region to be loaded without additional modifications and delivered into mammalian living systems in a manner independent of their size, isoelectric point. And this system could deliver various antibodies 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 antibodies in vivo. Therefore , the Nanoblock-Antibody Delivery Kit may be used in applications relevant to intercellular signaling, cell cycle regulation, apoptosis, oncogenesis and transcriptional regulation.

KIT CONTENTS

Nanoblock-Antibody Delivery Kit (NES002-01)

Catalog No.	Reagent	Quantity	Storage/Stability
NES002-01-01	$AuNP^{IgG}$ Reagent (25nM)	1 ml	4°C for 2 month
NES002-01-02	FITC-Mouse IgG	10 µl	-20°C for 2 month
NES002-01-03	10x Binding buffer	1 ml	RT for 1 year
NES002-01-04	PBS buffer	1 ml	RT for 1 year
NES002-01-05	$MgCl_2$ (0.1M)	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		 **$AuNP^{IgG}$ Reagent** Pre-incubate at 80 °C for 5 min and cool down at RT for 5 min	$AuNP^{IgG}$ Reagent (µl)	2	10	40
3		 **Antibody solution** - Prepare Antibody solution with PBS buffer and $MgCl_2$	$MgCl_2$ (0.1 M)	1	5	20
			PBS buffer (µl)	6	30	120
			5 µM Antibody (µl)	1	5	20
4		 **Conjugation** **[$AuNP^{IgG}$ - Antibody]** - Mix $AuNP^{IgG}$ reagent and Antibody solution - Incubate for 10-15 min at RT				
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 antibody 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^{IgG} / Antibody Conjugation

Protocol

1) Prepare AuNP^{IgG} reagent in microtube
- Pre-incubate the AuNP^{IgG} reagent at 80 °C for 5 min
- Cool down at RT for 5 min to refold the secondary structure of aptamer

2) Prepare antibody solution
- Adjust antibody concentration to 5 µM
- Make anbidody solution with PBS buffer and 0.1M $MgCl_2$ (0.1 M)

  [Note] High concentration of antibody can lead to precipitation. We recommend that final concentration of antibody should NOT be more than 100 µM.

3) Construct conjugates of AuNP^{IgG} and antibody
- Add AuNP^{IgG} reagent into antibody solution and gently tapping.
- Incubate 10-15 min at room temperature.

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

Experimental consideration

Table 2: Suggested amount of antibody and AuNP^{IgG}

Culture vessel	5 μM Antibody	$MgCl_2$ (0.1 M)	PBS buffer	AuNP^{IgG}	Total Volume
96 well	1 μL	1 μL	6 μL	2 μL	10 μL
24 well	5 μL	5 μL	30 μL	10 μL	50 μL
6 well	20 μL	20 μL	120 μL	40 μL	200 μL

** Because antibodies differ one from another, reflecting a variety of physical properties, conjugation efficiency of antibod* ies *with AuNP^{IgG} reagent are variable. So, users need to determine optimum conditions to deliver your antibody. If your sample is aggregate, add 10X pH Buffer instead of PBS Buffer.*

[Note] The presence of NaCl (>300 mM) or $MgCl_2$ (>10 mM) precipitate the AuNPs. If NaCl and $MgCl_2$ are present as high concentration in your 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 antibody as pure as possible. Otherwise, it can be removed by dialysis.*

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 antibody delivery efficiency (1-48 h). Incubation time will depend on your experimental purpose (for example , incubate 1~4 h to detect delivered antibody , and incubate over 24 h to determine cell toxicity of delivered antibody)

Experimental consideration

** AuNP^{IgG} reagent can be used onto cells in serum-free media. In this case, replace the complete culture medium by serum-free medium . This procedure can be more efficient to deliver certain antibodies in some cells . After 3-4 h, add some serum - containing medium if further incubation time is necessary.*

Appendix

1 QUALITY CONTROLS

2 TROUBLESHOOTING

1. Low delivery efficiency

Check point	Suggestion
Antibody purity	Make sure that the your antibody is highly pure and devoid of additives such as NaCl, $MgCl_2$ BSA or detergents.
$AuNP^{IgG}$ reagent amount	Optimize the quantity of $AuNP^{IgG}$ reagent as described in the table 2.
Antibody amount	Optimize the $AuNP^{IgG}$ / antibody ratio. We recommend using at least 20 times as much antibody as gold.
Cell density	A non-optimal cell density at the time of antibody 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 4h of incubation.
Incubation time	The optimal time range between delivery and assay varies with cells, type of antibody, kinetics of biological function, etc. The delivery efficiency can be monitored after 4 to 96h. FITC-Mouse IgG can be used to quantitatively monitored delivery kinetics.
Old $AuNP^{IgG}$ / antibody complexes	$AuNP^{IgG}$ reagent / antibody complexes must be freshly prepared every time. Complexes prepared and stored for more than 1 hour can be aggregated. Depending on the antibody, 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 FITC-Mouse IgG provided in the kit can be used as positive control for delivery efficiency.
$AuNP^{IgG}$ reagent storage	Delivery efficiency can slowly decrease if $AuNP^{IgG}$ reagent is kept more than one week at room temperature.

2. Cellular toxicity

Check point	Suggestion
Concentration of $AuNP^{IgG}$ / antibody too high	Decrease the amount of $AuNP_{IgG}$ / antibody complexes added to the cells by lowering the antibody amount or the $AuNP_{IgG}$ reagent. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined
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
Antibody is cytotoxic	Use suitable controls such as cells alone, $AuNP^{IgG}$ reagent alone or mock delivery (with FITC-Mouse IgG provided).
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.
Antibody quality	Use high quality antibody as impurities could lead to cell death.

3 EXAMPLE PROTOCOL AND RESULT

Delivery of FITC-Mouse IgG 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. Pre-incubate the $AuNP_{IgG}$ reagent at 80 °C for 5 min and cool down at RT for 5 min.
3. Mix 1.5 µl of FITC-Mouse IgG, 5 µl of $MgCl_2$ (0.1 M) and PBS buffer up to 40 µl.
4. Add 10 µl of pre-incubated $AuNP^{IgG}$ reagent into antibody, mix by gently tapping.
5. Incubate 10-15 min at room temperature.
6. Distribute mixture 5 onto the cells.
7. Incubate cells in a 5% CO_2 incubator at 37°C for 1-24 hours.
8. After the incubation, wash the cells twice with PBS and proceed with the appropriate assay.

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THANK YOU FOR CHOOSING

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