Paretor – Predictable Delivery Protocols & Formulations

Mineral Nanoparticle Colloid | Cell transfection | Cargo Carrier

TRACER INFORMATION (10-001→ 10-003)

Tracer Chemistry Overview:
Total amount in supplied aliquot: 3.32 nmol in 33 µL
Left side: Phosphonate ligand that binds the PA-001 iron oxide coordination shell
Middle: thiol-reactive linker (GSH-sensitive; intracellular cleavage expected)
Right side: thiolated AQuora fluorescein reporter (488/515 nm or 495/535 nm compatible)
Reporter stability: strong, non-quenching spacer; suited for FACS and live imaging

TRACER LOADING PROTOCOL

Purpose: To load the Paretor 10-001 particle surface with a defined number of fluorescein probes per particle using phosphonate–iron coordination and maleimide-compatible disulfide chemistry.

1. Tracer Dilution

Dilute Tracer in the following buffer:10 mM HEPES 150 mM NaCl pH 6.9. Two recommended dilution options depending on desired probe density:

Tracer Dilution	Add Volume	Expected Loading
100× dilution	25 µL	~ 20 probes/particle
20× dilution	25 µL	~ 200 probes/particle

Particle Input:
Use 7.5×10^{10} particles per loading reaction.

2. Conjugation Step

Combine diluted Tracer with PA-001 (10-001) particles. Mix gently (do not vortex).Incubate 30 minutes at 37 °C in the dark. Maintain low light conditions throughout (AQuora is highly stable but still light-responsive).This incubation yields predictable, reproducible surface loading.

3. Purification Step (Strongly Recommended)

Dialyze the loaded particles to remove unbound Tracer:
Dialysis buffer: 10 mM MES + 150 mM NaCl, pH 6.9. Use 10 kDa MWCO cellulose membrane
Two rounds of dialysis, each using 100× sample volume
Duration: 30 minutes each at room temperature, dark
This step significantly improves imaging clarity and reduces background fluorescence.

Thiolated constructs can be utilized off-the-shelf with Paretor PA-001 design. If thiol chemistry is not amenable to your project, custom linker systems can be made on request. Beyond small molecules and oligonucleotides PA-001 has the potential to carry proteins and polynucleotides up to 15kDa.

SUGGESTED READY-TO-LOAD RNA PROTOCOL for PA-001 (10-002; 10-003)

Your assay system may have different requirements. Suggestions are physiologically relevant starting points.

NOTE: Particles can be diluted to a concentration of $1x10^{11}$ particle/ml. Dilute in PBS pH 7.2-7.4

Purpose	Conjugation and *in vitro* dosing of thioloated oligonucleotides using PA-001 (10-002)
Format	12 well plate, 50,000-100,000 plated cells/well, 1ml volume
Target Outcome	Cytosolic delivery of ~2,000 siRNA oligos per cell (BT20, H69AR for example) over 2 hours
Assay Set Up	Suggest titration range of 5,000 to 100,000 particles per cell of PA-001 (10-002) particles and 0.1-0.6 pmol of oligo per test well
Conjugation	In a sterile amber 1.5ml tube combine PA-001 (10-002) carrier with Activator-treated thiolated oligo (purification-free thiol deprotection). Mix gently. Do not vortex. Incubate for 20 minutes at room temperature in dark. Proceed to dosing. Optional use of Scavenger to remove unbound oligo
Dosing	Add your range of activated PA-001 Carrier (10-002_ : oligo complex to cells. Mix gently
Dosing Considerations	The PA-001 Carrier (10-004) is designed to transfect a broad range of cell types expressing PDI, CD36, CD71 and EGFR or whose role is a sentinel. Optimal conditions may require titration of both PA-001 (10-002) carrier and oligo

CORE USE CASE BRIEF

Suggested use case

Product	PA-001 carrier (10-002) **NOTE:** The in *vivo* portion of this use case requires concentrated construct available on request
Application	Oligonucleotide delivery in rapidly dividing cancer cells
Model System	4T1 cell line & Balb/c orthotopic breast cancer model
Rationale	Over the past two decades, iron oxide nanoparticles (IONPs) have been extensively studied and deployed in oncology-focused drug delivery, particularly for passive accumulation in RES-rich tissues (e.g., liver, spleen, lung, lymph nodes), tumor penetration via EPR/Wharburg effects, and cytosolic delivery of oligonucleotide payloads (siRNA, miRNA, ASO)
Use Case	Evaluation of RNA approaches in 4T1 mouse breast cancer cell line followed by progression to the orthotopic 4T1 model in Balb/c mice
In *Vitro*	Assess viability, transfection efficiency, functional efficacy of PA-001 carrier-delivered RNA
In Vivo	Intravenous administration in Balb/c 4T1 tumor-bearing mice. Assess biodistribution, tumor accumulation and functional readouts