Non-viral, non-lipid peptide nanoparticle gene delivery

PRE-FORMULATED PARTICLE PROTOCOL

Directions for use of pre-formed NCPP particles supplied ready-to-dose.

1 Product identity

Product / particle name	NCPP
Peptide	2070X, 2904X (or as specified)
PEG	2K Zn-PEG
Cargo construct	pDNA (GFP, CFTR, etc. as labelled)
Lot / batch no.	_____
pDNA concentration	~400 µg/mL (dosing basis)
Particle size (Z-Ave, DLS)	~100 nm (Malvern Zetasizer)
Polydispersity index (PDI)	≤ 0.2

2 Composition & release specifications

CRITICAL PRINCIPLE — CELL-SPECIFIC DOSE OPTIMISATION
THERE IS NO UNIVERSAL OPTIMAL DOSE.

Different cell types have different nanoparticle uptake, intracellular trafficking and tolerance, so the optimal NCPP dose may vary. The doses below are starting points, not universal optima.

For each new cell model, test at least three doses appropriate to the cargo and assess both delivery efficiency and cell viability. A negative result at one dose does not establish that the formulation is ineffective. Record the dose, exposure conditions and readout time.

The pDNA concentration is the basis for dosing. Dose on pDNA mass — do not back-calculate from peptide or PEG content.

3 Directions for use

NCPPs are supplied pre-formulated and ready for dosing. Application conditions differ between conventional submerged cultures and ALI models; use the relevant section below.

Before dosing (all cell types):

Equilibrate. Bring the vial to room temperature before opening. Invert gently 3–4 times to mix. Do not vortex vigorously and do not centrifuge the particles.

Do not dilute in incompatible water. If dilution is required, use ultrapure water only. Do NOT use MilliQ (residual ions screen the peptide–DNA interaction) or TE buffer (Tris/EDTA screen complexation and EDTA chelates the Zn).

3A Immortalized cell lines (HEK293T, HeLa, Jurkat, etc.)

Standard adherent or suspension cultures in liquid medium.

12-well plate: Standard protocol

- Example starting dose: 20 µg DNA per well (not a universal optimum; run a dose-response screen)
- Prepare transfection mix: Add 50 µL NP stock (400 µg/mL) to 350 µL OptiMem
- To OptiMem: use OptiMem containing 0.05% HSA final concentration (prepared as described in §6)
- Vortex and sit for 5 minutes at room temperature
- Remove culture medium; add transfection mix to cells
- Incubate: 3 to 4 hours at 37°C, 5% CO_2
- Remove transfection mix; add 1 mL fresh serum-containing medium

96-well plate: Low-volume format

Standard well volume: 100 µL medium per well

- Example dose range: 4–8 µg DNA per well (not a universal optimum; include lower doses for sensitive models)
- Calculate volume: (desired µg ÷ 400 µg/mL) × 1000 = µL stock per well
- Prepare OptiMem with 0.05% HSA as above
- Mix NP with OptiMem/HSA, vortex and sit 5 minutes
- Incubate: 3 to 4 hours at 37°C, 5% CO_2
- Remove transfection mix; add 100 µL fresh serum-containing medium

3B ALI (Air-Liquid Interface) cultures

Differentiated epithelial cultures (e.g., primary or iPSC-derived respiratory epithelium). ALI cultures have an air-exposed apical surface (minimal fluid, mucus layer only) and a submerged basolateral chamber.

ALI dosing principles:

Dose should be expressed as pDNA-equivalent per insert and interpreted together with the exposed apical surface area. Dose, application volume and exposure period may require optimisation for the specific ALI model.

The example below is a practical starting condition for a 4 µg dose and should not be interpreted as a universal optimum.

Approximate exposed surface area for a typical 24-well insert: ~0.33 cm^2.

Example ALI protocol — 4 µg pDNA-equivalent dose (24-well format):

For a 4 µg pDNA-equivalent dose, use the equivalent of 10 µL NCPP particle stock at ~400 µg/mL per insert.

A typical preparation is 20 µL particle stock made up to 50 µL total transfection mix using OptiMem containing 0.05% HSA; apply 25 µL of the prepared mix per insert. This delivers the equivalent of 10 µL particle stock, or approximately 4 µg pDNA, per insert.

Mix gently and sit for 5 minutes at room temperature before application.

Apply the prepared transfection mix gently to the apical surface.

Typical exposure: approximately 5.5 hours at 37°C, 5% CO_2. Exposure time may require optimisation for the model.

Aspirate residual transfection mix from the apical surface with gentle pipetting after the exposure period.

Basolateral medium remains unchanged throughout transfection.

- **Post-transfection:**
- Typical readout: 72 hours post-dosing, or as appropriate for the cargo and experimental model.

Imaging: fluorescence microscopy or other validated expression readout. Flow cytometry may be used where cells are dissociated and the assay has been established.

4 Optional: Repeat transfection protocol

Repeat dosing is straightforward to perform and is supported by prior NCPP experimental experience. Where appropriate for the cell model, a second exposure 24–48 hours after the first can be evaluated using the same peptide formulation. Sequential dosing with a different peptide formulation is an additional experimental option and has not been validated as superior for all cell types. Monitor cell viability and optimise dose and exposure conditions.

Example: dose on Day 0 and re-dose on Day 1 or Day 2; assess expression and viability at timepoints appropriate for the cargo and model. For a repeat dose, reapply the relevant dosing and medium-replacement steps in §3. Repeat dosing is an optimisation variable, not a universal requirement.

5 Recommended controls

- Untreated cells (autofluorescence baseline)
- Your lab's current transfection method run in parallel (direct comparison)
- Naked plasmid DNA control (in OptiMem ± HSA)
- Vehicle-only / particle-free control as applicable

6 Human serum albumin (HSA) preparation & use

HSA use depends on the application. For in vitro cell culture, HSA is added to the warmed transfection medium first, to a final concentration of 0.05% w/v, before the nanoparticles are slowly added. Do not add HSA directly to the supplied NCPP vial for routine in vitro use. Direct addition of HSA to nanoparticles is a separate procedure used for in vivo administration and is not covered by this in vitro protocol.

Preparation:

- Prepare 0.5% HSA stock: Dissolve 50 mg HSA in 10 mL ultrapure water. Store at 4°C; prepare fresh as required.

OptiMem transfection medium (0.05% HSA):

- Add 100 µL of 0.5% HSA stock to 900 µL OptiMem to make 1 mL at 0.05% HSA
- OR: Add 500 µL of 0.5% HSA stock to 4.5 mL OptiMem to make 5 mL at 0.05% HSA
- Mix by gentle inversion (do not vortex OptiMem alone)
- Use within 24 hours of preparation

In vitro: adding NP to HSA-containing transfection medium:

- Prepare the OptiMem/0.05% HSA mixture first, then slowly add the required volume of NP
- Mix gently and sit for 5 minutes at room temperature before adding to cells
- Apply promptly after the 5-minute equilibration period.

In vitro buffers: If particles are diluted into a buffer such as HBSS rather than culture medium, a higher HSA concentration may be required to maintain particle stability; HSA concentrations up to 0.3% w/v may be required. Optimise for the buffer and application.

Biosafety: HSA is human-derived. Handle per institutional biosafety requirements for human-origin material.

7 Storage

Upon receipt, store particles at 4 °C immediately. DO NOT FREEZE. Protect from prolonged heat and direct light.

Shelf life from date of formulation: 3 months at 4 °C. Particles are stable during shipping at ambient temperature (15–25 °C); however, storage at 4 °C extends usable life and is recommended.

Note: Diluted or mixed particles should be used within 1 hour of preparation.

8 Status & limitations

NON-GMP — FOR RESEARCH USE ONLY. Not manufactured under GMP. Not for human, clinical, diagnostic or therapeutic use. Supplied for in vitro research evaluation only.

Provided without warranty as to performance in any specific cell type. Material remains the property of Loxegen Holdings Pty Ltd; onward transfer and publication subject to a separate material transfer agreement.

9 Document information

Document No.	NCPP002
Version	3.7
Effective date	10/11/2026
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