

Nanocin™-SM

Small molecule DELIVERY PROTOCOL

for Tecrea Ltd products:

TNSM-250

TNSM-500

Product information

Nanocin™-SM is a novel nanoparticle-based delivery platform that efficiently delivers non-cell-permeable small molecules into a range mammalian cell.

For research use only.

Quality control

Each batch is tested using biophysical methods and by ensuring efficient delivery of a non-cell-permeable green fluorescent DNA ligand (SytoxGreen) into HeLa cells, assessed by fluorescent microscopy and flow cytometry.

Shipping, storage and shelf life

Nanocin™-SM products are shipped at room temperature, stored at 4°C and are stable for at least one year. The expiry date is indicated on the tube label.

Safety

Nanocin™-SM shows very low toxicity in a range of assays. See MSDS for more details and handling instructions.

www.tecrea.co.uk/support/MSDS

Technical support & scientific advice

Tecrea Ltd provides extensive technical support and we are pleased to offer scientific advice for your experiments.

Please contact us at: info@tecrea.co.uk

Technical resources

FAQs at: www.tecrea.co.uk/suport/FAQs

Troubleshooting guide: www.tecrea.co.uk/support

© **TOP TIP #1** See our *rapid* protocol, which saves at least one day of time, several steps and reagents.

nanocin™
SM

Transfection and Cell Delivery
From lab to clinic

tecraa™
creative cell & tissue delivery

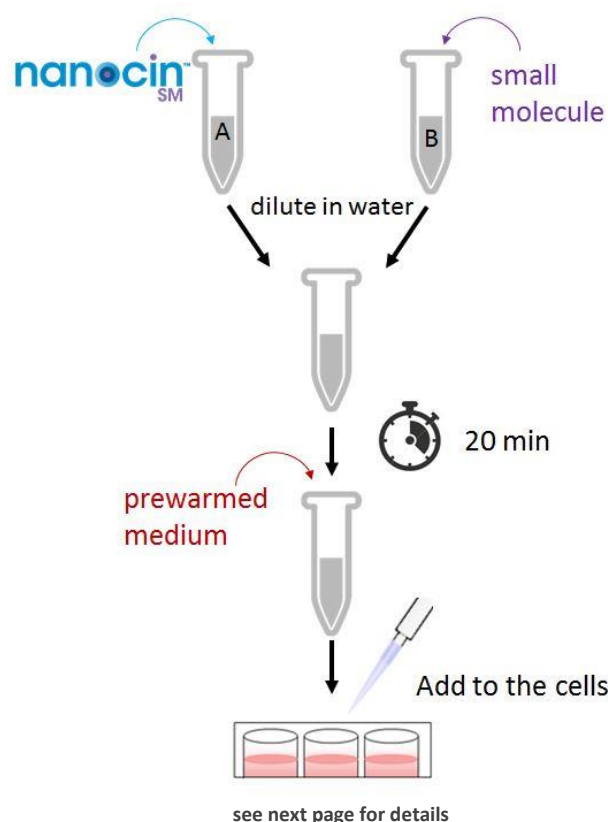
Contents and ordering

Cat #	Reagent volumes	Number of transfections (12-well plate)
TNSM-250	0.25 ml Nanocin™-SM	50-75
TNSM-500	0.5 ml Nanocin™-SM	100-150

Related products

Product	Cat #
Nanocin™-RNAi	TNR-250
	TNR-500
	TNR-1000
Nanocin™-plasmid	TNP-250
	TNP-500
	TNP-1000
Nanocin™-PRO (for protein & peptide delivery)	TNPRO-250
	TNPRO-500

Protocol overview



STANDARD SMALL MOECULE DELIVERY PROTOCOL

Use this protocol to delivery impermeable small molecules into mammalian cells after the cells have recovered from splitting or seeding. The details here are for a **12-well** plate format. For other formats, see table below. All volumes are given per well.

SET-UP

- Seed and grow cells to 60-80% confluence.
- Vortex Nanocin™-SM for 10 seconds and centrifuge briefly
- Freshly dilute a cargo molecule (e.g. SytoxGreen) to 1mg/ml in water, or a suitable alternative solvent (see below).
Protect from direct light.

START delivery experiment

1. Prepare transfection mixture for 12 well plate (example):

- **Tube A** → Dilute 2 µl of 1 mg/ml cargo molecule with water to a final volume of 50 µl, mix thoroughly [adjust pipette to 50 µl and pipette the full volume up and down 5-10 times]
- **Tube B** → Dilute 4 µl of Nanocin™-SM reagent with water to a final volume of 50 µl, mix thoroughly [adjust pipette to 50 µl and pipette the full volume up and down 5-10 times]
- Transfer the solution from tube A into tube B, mix thoroughly [adjust pipette to 100 µl and pipette the full volume up and down 5-10 times]. Incubate for 20 minutes at room temperature.

2. Cell delivery:

- Add 900 µl of pre-warmed growth medium to each tube prepared in step 1, mix thoroughly [adjust pipette to 1 ml and pipette the full volume up and down 5-10 times].
- Remove old growth media from wells. Immediately add diluted cell delivery mixture, by pipetting gently onto well walls.
- Incubate for approximately 2 hours and then process for microscopy. (For cell phenotype assessment, the delivery mixture can include within the cell culture for several days, as required.)

RAPID SMALL MOECULE DELIVERY PROTOCOL

Use this *rapid* protocol to transfect protein or peptide into mammalian cells at the time of splitting or seeding. The *rapid* protocol saves at least one day and several steps☺. The details here are for a **12-well** plate format. For other formats, see table below. All volumes given are per well.

SET-UP

- Vortex Nanocin™-SM for 10 seconds and centrifuge briefly
- Freshly dilute a cargo molecule (e.g. SytoxGreen) to 1mg/ml in water, or a suitable alternative solvent (see below).
Protect from direct light.

START delivery experiment

1. Prepare transfection mixture for 12 well plate (example):

- **Tube A** → Dilute 2 µl of 1 mg/ml cargo molecule with water to a final volume of 50 µl, mix thoroughly [adjust pipette to 50 µl and pipette the full volume up and down 5-10 times]
- **Tube B** → Dilute 4 µl of Nanocin™-SM reagent with water to a final volume of 50 µl, mix thoroughly [adjust pipette to 50 µl and pipette the full volume up and down 5-10 times]
- Transfer the solution from tube A into tube B, mix thoroughly [adjust pipette to 100 µl and pipette the full volume up and down 5-10 times]. Incubate for 20 minutes at room temperature.

[While the transfection mixture incubates, trypsinise your cells and prepare suspensions in growth medium at approximately 4×10^5 cells/ml, then add 500 µl to each well (1/2 of final volume in well).]

2. Cell delivery:

- Add 400 µl of pre-warmed growth medium (9x volume of transfection mixture) to each tube prepared in step 1, mix thoroughly and then add drop-by-drop to wells with a gentle swirl of the plate to mix.
- Incubate for approximately 2 hours and then process for microscopy. (For cell phenotype assessment, the delivery mixture can include within the cell culture for several days, as required.)

Plate	Well surface area	Media (vol/well)	Transfection mixture volume	Fresh media volume	small molecule delivery	
					small molecule (1 mg/ml)	Nanocin™-SM
24-well	2 cm ²	500 µl	50 µl	450 µl	1 µl	2 µl
12-well	4 cm ²	1 ml	100 µl	900 µl	2 µl	4 µl
6-well	10 cm ²	2.5 ml	250 µl	2250 µl	5 µl	10 µl
60-mm	20 cm ²	5 ml	500 µl	4500 µl	10 µl	20 µl

Notes:

- growth medium may be with or without FCS and antibiotics
- use transfection mixture within 60 minutes after preparation; mix thoroughly at all mixing steps by pipetting up & down the full volume
- amount of small molecule or Nanocin™-SM used can be varied +/- 50% to optimize
- if your cargo molecule is not soluble in water, feel free to contact us for guidance on selection of a suitable alternative solvent

TOP TIP #2 Nanocin™-SM products have such low toxicity that researchers can design experiments that involve multiple, serial delivery, and small molecule delivery into cultures with low cell densities is possible.

TOP TIP #3 Nanocin™-SM products are for research uses only, but Tecrea's technology is compatible with clinical development, so you can envision taking your research program from the lab to clinic. Just ask us for more information.