



# Small molecule delivery protocol

# FOR TECREA PRODUCTS TNSM-250 AND 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/HEK293T cells, assessed by fluorescent microscopy and flow cytometry.

## Shipping, storage and shelf life

**Nanocin** products are shipped at room temperature, stored at 2-8°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 our MSDS for more details and handling instructions. https://www.tecrea.com/product/nanocin-sm/

# Technical resources and scientific advice

Tecrea provides extensive technical support and we are pleased to offer scientific advice for your experiments. Please contact us for more information. info@tecrea.com / Frequently asked questions

# **Helpful information**

Save time and increase experiment efficiency with **Nanocin SM's** rapid protocol (see next page).

Nanocin SM products are effectively non-toxic, meaning they can facilitate multiple deliveries. Small molecule delivery into cultures with low cell densities is also possible.

Nanocin SM products are for research use only, but they are also highly compatible with clinical development, meaning you can carry research from lab to clinic with confidence.

# **Contents and ordering**

Unit size (mL)	Reactions*	Cat No.
0.25	50-75	TNSM-250
0.5	100-150	TNSM-500

<sup>\*</sup>Approximate number based on 12-well plate

# **Related products**

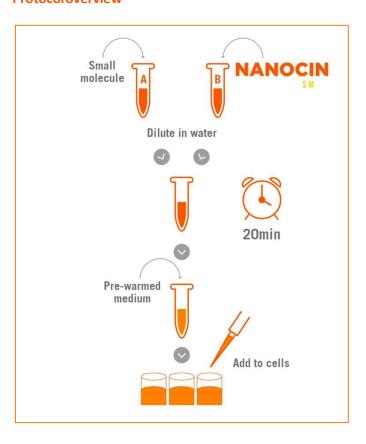
roduct	Cat No.

**Nanocin RNA** TNR-250, TNR-500, TNR-1000

**Nanocin PLASMID** TNP-250, TNP-500, TNP-1000, TNP-10000

Nanocin PRO TNPRO-250, TNPRO-500 (for small molecule delivery)

# **Protocol overview**



# Standard SMALL MOLECULE DELIVERY PROTOCOL

# Rapid SMALL MOLECULE DELIVERY PROTOCOL

Use this protocol to deliver 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.

Use this rapid protocol to deliver impermeable small molecules 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

- 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.

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# **START SM delivery**

### Step 1. Prepare reaction mixture for 12-well plate (example):

**Tube A** Dilute 2  $\mu$ l of 1 mg/ml cargo molecule with water, creating a final volume of 50  $\mu$ l. Mix thoroughly by pipetting the full volume up and down 5-10 times. **Tube B** Dilute 4  $\mu$ l of **Nanocin SM** reagent with water, creating a final volume of 50  $\mu$ l. Mix thoroughly by pipetting the full volume up and down 5-10 times. Transfer the solution from tube A into tube B, and mix thoroughly by adjusting pipette to 100  $\mu$ l and pipetting the full volume up and down 5-10 times. Incubate for 20 minutes at room temperature.

Step 2. Cell delivery: Add 900  $\mu$ l of pre-warmed growth medium to each tube prepared in step 1, then mix thoroughly by pipetting 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.)

# **START SM delivery**

# Step 1. Prepare reaction mixture for 12 well-plate (example):

**Tube A** Dilute 2  $\mu$ l of 1 mg/ml cargo molecule with water, creating a final volume of 50  $\mu$ l. Mix thoroughly by pipetting the full volume up and down 5-10 times. **Tube B** Dilute 4  $\mu$ l of **Nanocin SM** reagent with water, creating a final volume of 50  $\mu$ l. Mix thoroughly by pipetting the full volume up and down 5-10 times. Transfer the solution from tube A into tube B, and mix thoroughly by adjusting pipette to 100  $\mu$ l and pipetting the full volume up and down 5-10 times. Incubate for 20 minutes at room temperature. While the reaction mixture incubates, trypsinise your cells and prepare suspensions in growth medium at approximately  $4x10^5$  cells/ml, then add 500  $\mu$ l to each well (half of final volume in well).

**Step 2. Cell Delivery:** Add 400  $\mu$ l of pre-warmed growth medium to each tube prepared in step 1, then <u>mix thoroughly</u>. Add drop-by-drop to wells with a gentle swirl of the plate to mix. Incubate for approximately two hours and then process for microscopy. (For cell phenotype assessment, the delivery mixture can include within the cell culture for several days, as required.)

# Alternative volumes for other plates' formats

Plate	Well surface area	Media (Vol/Well)	Reaction mix volume	Fresh media volume	Small molecule deliv	Small molecule delivery	
					Small molecule (1mg/ml)	Nanocin SM	
24-well	$2\ cm^2$	500 μΙ	50 μΙ	450 μΙ	1 μΙ	2 μΙ	
12-well	4 cm <sup>2</sup>	1 ml	100 μΙ	900 μΙ	2 μΙ	4 μΙ	
6-well	10 cm²	2.5 ml	250 μΙ	2250 μl	5 μΙ	10 μΙ	
60-mm	$20\mathrm{cm}^2$	5 ml	500 μΙ	4500 μΙ	10 μΙ	20 μΙ	

# Notes:

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

