



# Protein/peptide delivery protocol

FOR TECREA PRODUCTS TNPRO-250 AND TNPRO-500

## Product information

**Nanocin PRO** is a novel nanoparticle-based platform that can efficiently deliver proteins and peptides into a range of mammalian cells. **For research use only.**

## Quality control

Each batch of **Nanocin PRO** is tested using biophysical methods and by ensuring efficient delivery of R-phycoerythrin into HeLa cells, assessed by fluorescence microscopy.

## Shipping, storage and shelflife

**Nanocin** products are shipped at room temperature and stored at 2-8°C and are stable for at least one year. The expiry date is indicated on the tube label.

## Safety

**Nanocin PRO** products show very low toxicity in a range of assays. See our MSDS for more details and handling instructions.

<https://www.tecrea.com/product/nanocin-pro/>

## 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](mailto:info@tecrea.com) / [Frequently asked questions](#)

### Helpful information

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

**Nanocin PRO** products are effectively non-toxic, meaning they can facilitate multiple reactions.

**Nanocin PRO** 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

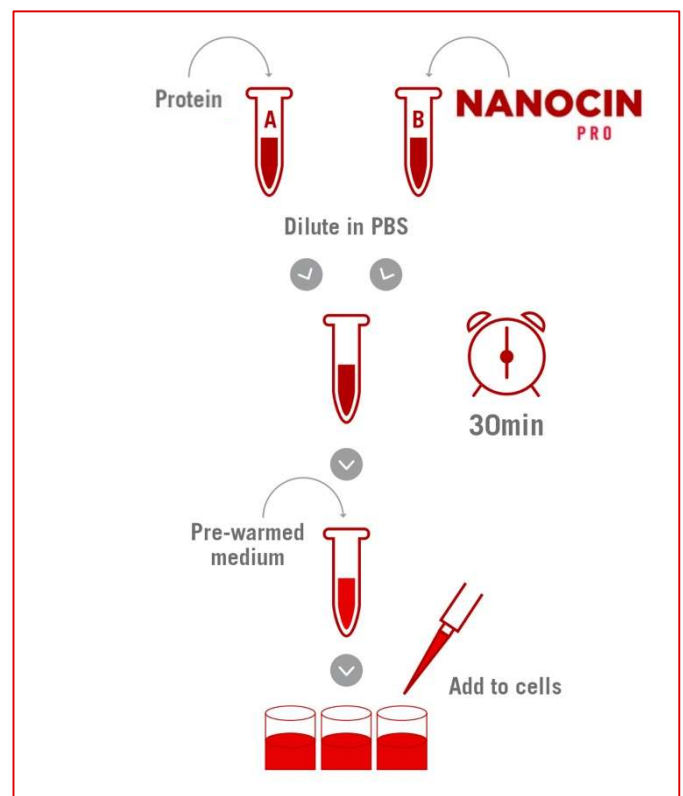
Unit size (mL)	Reactions*	Cat No.
0.25	50-75	TNPRO-250
0.5	100-150	TNPRO-500

\*Approximate number based on 12 well plate

## Related products

Product	Cat No.
<b>Nanocin RNA</b>	TNR-250, TNR-500, TNR-1000
<b>Nanocin PLASMID</b>	TNP-250, TNP-500, TNP-1000, TNP-10000
<b>Nanocin SM</b> (for small molecule delivery)	TNSM-250, TNSM-500

## Protocol overview



# Standard

## PROTEIN/PEPTIDE DELIVERY PROTOCOL

Use this protocol to deliver protein or peptide 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 (for low-confluence experiments see notes below\*).
- Vortex **Nanocin PRO** for 10 seconds and centrifuge briefly

### START Protein delivery

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

**Tube A** Dilute 4 µl of 1 mg/ml protein or peptide with PBS, creating a final volume of 50 µl. Mix thoroughly by pipetting the full volume up and down 5-10 times. **Tube B** Dilute 4 µl of **Nanocin PRO** reagent with PBS, creating a final volume of 50 µl. 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 pipetting the full volume up and down 5-10 times. Incubate for 30 minutes at room temperature.

**Step 2. Cell Delivery:** Add 900 µl 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 two hours and then process for microscopy.

# Rapid

## PROTEIN/PEPTIDE DELIVERY PROTOCOL

Use this rapid protocol to deliver protein or peptide into mammalian cells at the time of splitting or seeding. The rapid protocol saves at least one day of experiment time and reduces the process by 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 PRO** for 10 seconds and centrifuge briefly.

### START Protein delivery

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

**Tube A** Dilute 4 µl of 1 mg/ml protein or peptide with PBS, creating a final volume of 50 µl. Mix thoroughly by adjusting pipette to 50µl and pipetting the full volume up and down 5-10 times. **Tube B** Dilute 4 µl of **Nanocin PRO** reagent with PBS, creating a final volume of 50 µl. 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 pipetting the full volume up and down 5-10 times. Incubate for 30 minutes at room temperature. While the reaction mixture incubates, trypsinise your cells and prepare suspensions in growth medium at approximately  $4 \times 10^5$  cells/ml, then add 500 µl to each well (half of final volume in well).

**Step 2. Cell Delivery:** Add 400 µl of pre-warmed growth medium to each tube prepared in step 1, then mix thoroughly. 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.

### Alternative volumes for othe plates' formats

Plate	Confluence	Well surface area	Media (Vol/Well)	Reaction mix volume	Fresh media volume	Protein/peptide delivery	
						Protein or peptide (1 mg/ml)	Nanocin PRO
24-well	30-60%*	2 cm <sup>2</sup>	500 µl	38 µl	462 µl	1.5 µg	1.1 µl
	60-80%	2 cm <sup>2</sup>	500 µl	50 µl	450 µl	2 µg	1.5 µl
12-well	30-60%*	4 cm <sup>2</sup>	1 ml	75 µl	925 µl	3 µg	2.3 µl
	60-80%	4 cm <sup>2</sup>	1 ml	100 µl	900 µl	4 µg	3 µl
6-well	30-60%*	10 cm <sup>2</sup>	2.5 ml	188 µl	2312 µl	7.5 µg	5.6 µl
	60-80%	10 cm <sup>2</sup>	2.5 ml	250 µl	2250 µl	10 µg	7.5 µl
60-mm	30-60%*	20 cm <sup>2</sup>	5 ml	375 µl	4625 µl	15 µg	11.3 µl
	60-80%	20 cm <sup>2</sup>	5 ml	500 µl	4500 µl	20 µg	15 µl

#### 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 up and down the full volume