Nanocin™-PRO

Protein DELIVERY PROTOCOL for Tecrea Ltd products:

TNPRO-250
TNPRO-500

Transfection and Cell Delivery
From lab to clinic

Product information

Nanocin™-PRO is a novel nanoparticle-based delivery platform that cans efficiently delivery 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 shelf life

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

Safety

Nanocin™ products show 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** The *rapid* transfection protocol (next page) provides high transfection/delivery efficiencies and saves at least one day of time, several steps and reagents.

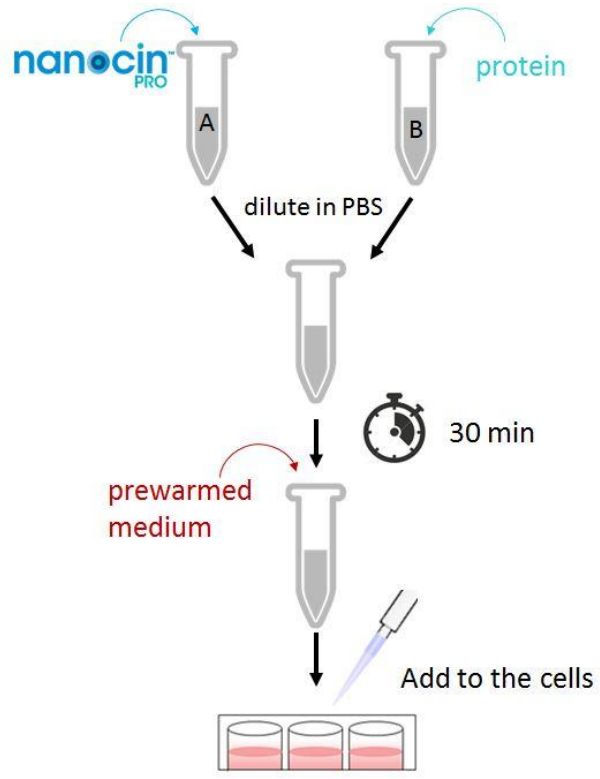
Contents and ordering

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

Related products

Product	Cat #
Nanocin™-RNAi	TNR-250 TNR-500 TNR-1000
Nanocin™-plasmid	TNP-250 TNP-500 TNP-1000
Nanocin™-SM (for small molecule delivery)	TNSM-250 TNSM-500

Protocol overview



see next page for details

STANDARD
PROTEIN/PEPTIDE DELIVERY PROTOCOL

Use this protocol to delivery 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 transfection

1. Prepare transfection mixture for 12 well plate (example):
- **Tube A** →Dilute 4 µl of 1 mg/ml protein or peptide with PBS 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 3 µl of Nanocin™-PRO reagent with PBS 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 30 minutes at room temperature.

2. Transfect:
- 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 transfection mixture, by pipetting gently onto well walls
- Incubate for approximately 2 hours and then process for microscopy.

RAPID
PROTEIN/PEPTIDE 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™-PRO for 10 seconds and centrifuge briefly

START transfection

1. Prepare transfection mixture for 12 well plate (example):
- **Tube A** →Dilute 4 µl of 1 mg/ml protein or peptide with PBS 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 3 µl of Nanocin™-PRO reagent with PBS 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 30 minutes at room temperature.
-While the transfection mixture incubates, trypsinise your cells and prepare suspensions in growth medium at approximately $4x10^5$ cells/ml, then add 500 µl to each well (1/2 of final volume in well).

2. Transfect:
- 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.

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

TOP TIP #2 Nanocin™-PRO products have such low toxicity that experiments can involve multiple, serial transfections

TOP TIP #3 Nanocin™-PRO 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.