Nanocin™-PLASMID

plasmid TRANSFECTION PROTOCOL for Tecrea Ltd products:

TNP-250
TNP-500
TNP-1000

Transfection and Cell Delivery
From lab to clinic

Product information

Nanocin™-plasmid is a novel transfection reagent dedicated to the efficient and non-toxic transfection into a range of mammalian cells, including primary cells and other sensitive cells. *For research use only.*

Quality control

Each batch is tested using biophysical methods and by ensuring efficient delivery of GFP encoding plasmid into HeLa cells, assessed by both microscopy and flow cytometry.

Shipping, storage and shelf life

Nanocin™-plasmid 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™-plasmid 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 and 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 efficiencies and saves at least one day of time, several steps and reagents.

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

TOP TIP #3 Nanocin™-plasmid 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 – the translational pathway. Just ask us for more information.

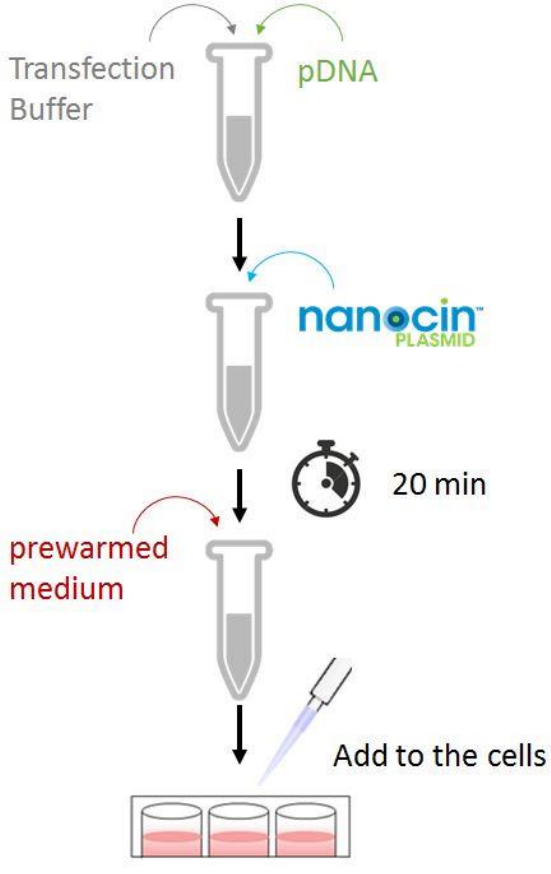
Contents and ordering

Cat #	Reagent volumes	Number of transfections (12-well plate)
TNP-250	0.25 ml Nanocin™-plasmid	50-75
TNP-500	0.5 ml Nanocin™-plasmid	100-150
TNP-1000	1.0 ml Nanocin™-plasmid	200-300

Related products

Product	Cat #
Nanocin™-RNAi	TNR-250 TNR-500 TNR-1000
Nanocin™-PRO (for protein & peptide delivery)	TNPRO-250 TNPRO-500
Nanocin™-SM (for small molecule delivery)	TNSM-250 TNSM-500

Protocol overview



see next page for details

STANDARD
PLASMID TRANSFECTION PROTOCOL

Use this protocol to transfect plasmid DNA 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™-plasmid reagent for 10 seconds and centrifuge briefly. Place reagent on ice.

START transfection

1. Prepare transfection mixture for 12 well plate (example):
- Dilute 1 µg plasmid DNA in Transfection Buffer to a final volume of 47.5 µl, mix thoroughly [adjust pipette to 50 µl and pipette the full volume up and down 5-10 times]. Place tube on ice.
- Add 2.5 µl of Nanocin™-plasmid reagent (50 µl total volume), mix thoroughly [pipette the full volume up and down 5-10 times].
- Incubate for 20 minutes on ice.

2. Transfect:
- Transfer tubes from ice to rack at room temperature. Add 950 µl of pre-warmed growth medium to each tube prepared in step 1 (1000 µl total volume), mix thoroughly.
- Remove old growth media from wells.
- Immediately add diluted transfection mixture by pipetting gently onto well walls.
- Incubate plates as usual for 24 - 72 hours.

RAPID
PLASMID TRANSFECTION PROTOCOL

Use this *rapid* protocol to transfect plasmid 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™-plasmid reagent for 10 seconds and centrifuge briefly. Place reagent on ice.

START transfection

1. Prepare transfection mixture for 12 well plate (example):
- Dilute 1 µg plasmid DNA in Transfection Buffer to a final volume of 47.5 µl, mix thoroughly [pipette the full volume up and down 5-10 times]. Place tube on ice.
- Add 2.5 µl of Nanocin™-plasmid reagent (50 µl total volume), mix thoroughly [pipette the full volume up and down 5-10 times].
- Incubate for 20 minutes on ice.

-While the transfection mixture incubates, prepare a cell suspension in growth medium at $4x10^5$ cells/ml (trypsinise first if necessary) then add 500 µl of suspended cells to each well.

2. Transfect:
- Transfer tubes from ice to rack at room temperature. Add 450 µl pre-warmed growth medium to each tube prepared in step 1 (500 µl total volume), mix thoroughly.
- Add drop-by-drop to cells in the well - gently swirl the plate to mix (1 ml final volume).
- Incubate plates as usual for 24 - 72 hours.

Plate	Confluence*	Well surface area	Media (vol/well)	Transfection mixture volume	Fresh media volume	Plasmid transfection	
						pDNA	Nanocin™-plasmid
24-well	30-60%*	2 cm^2	500 µl	19 µl	481 µl	0.38 µg	0.94 µl
	60-80%	**2 cm^2**	**500 µl**	**25 µl**	**475 µl**	**0.5 µg**	**1.25 µl**
12-well	30-60%*	4 cm^2	1 ml	38 µl	962 µl	0.75 µg	1.88 µl
	60-80%	**4 cm^2**	**1 ml**	**50 µl**	**950 µl**	**1 µg**	**2.5 µl**
6-well	30-60%*	10 cm^2	2.5 ml	94 µl	2406 µl	1.88 µg	4.69 µl
	60-80%	**10 cm^2**	**2.5 ml**	**125 µl**	**2375 µl**	**2.5 µg**	**6.25 µl**
60-mm	30-60%*	20 cm^2	5 ml	188 µl	4812 µl	3.8 µg	9.4 µl
	60-80%	**20 cm^2**	**5 ml**	**250 µl**	**4750 µl**	**5 µg**	**12.5 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 5-10 times