Nanocin™-RNAi

siRNA TRANSFECTION PROTOCOL for Tecrea Ltd products:

TNR-250
TNR-500
TNR-1000

Transfection and Cell Delivery From lab to clinic

Product information

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

Quality control

Each batch of Nanocin™-RNAi is tested using biophysical methods and by ensuring efficient delivery of siRNAs into HeLa cells, assessed by qRT-PCR.

Shipping, storage and shelf life

Nanocin™ 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™-RNAi 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 and scientific advice

Tecrea Ltd provides extensive technical support, and we are pleased to offer technical 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™-RNAi products have such low toxicity that experiments can involve multiple, serial transfections

TOP TIP #3 Nanocin™-RNAi 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 medicine pathway. Just ask us for more information.

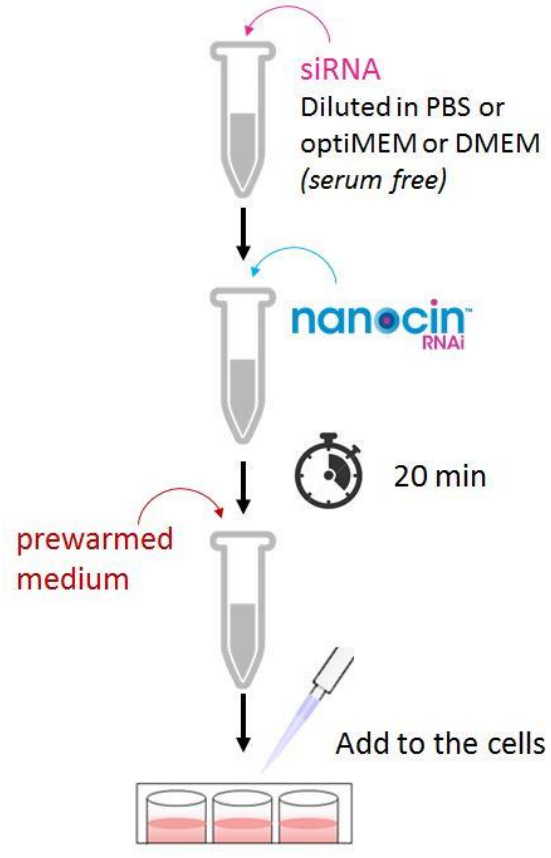
Contents and ordering

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

Related products

Product	Cat #
Nanocin™-plasmid	TNP-250 TNP-500 TNP-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
siRNA TRANSFECTION PROTOCOL

Use this protocol to transfect mammalian cells after the cells have recovered from splitting or seeding. The details here are for a **12-well** plate format and 20 nM final siRNA concentrations. For other formats, see table below. All volumes are given per well.

SET-UP

- Seed and grow cells to 60-80% confluence
- Vortex Nanocin™-RNAi reagent for 10 seconds and centrifuge briefly.

START transfection

1. Prepare transfection mixture for 12 well plate (example):
-Dilute 20 pmol of siRNA in PBS, optiMEM or DMEM (without serum) to a final volume of 46 µl, mix thoroughly [adjust pipette to 50 µl and pipette the full volume up and down 5-10 times]
- Add 4 µl of Nanocin™-RNAi reagent, mix thoroughly [pipette full volume up and down 5-10 times]
- Incubate for 20 minutes at room temperature.

2. Transfect:
-Add 950 µl of pre-warmed growth medium to each tube prepared in step 1 (1000 µl total), mix thoroughly [pipette full volume up and down 5-10 times]
-Remove old growth media from wells. Immediately add diluted transfection mixture, by pipetting onto well walls, with a gentle swirl of the plate to mix.
- Incubate plates as usual for 24 - 72 hours.

RAPID
siRNA TRANSFECTION PROTOCOL

Use this *rapid* protocol to transfect 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™-RNAi reagent for 10 seconds and centrifuge briefly.

START transfection

1. Prepare transfection mixture for 12 well plate (example):
-Dilute 20 pmol of siRNA in PBS, optiMEM or DMEM (without serum) to a final volume of 46 µl, mix thoroughly [adjust pipette to 50 µl and pipette the full volume up and down 5-10 times]
- Add 4 µl of Nanocin™-RNAi reagent, mix thoroughly [adjust pipette to 50 µl and pipette the full volume up and down 5-10 times]
- Incubate for 20 minutes at room temperature.

[While the transfection mixture incubates, prepare a cell suspension in growth medium at approximately $4x10^5$ cells/ml (trypsinise first if necessary), then add 500 µl to each well (1/2 of final volume in well)]

2. Transfect
- Add 450 µl of pre-warmed growth medium to each tube prepared in step 1 (500 µl total), mix thoroughly [pipette to 50 µl full volume up and down 5-10 times]. Add drop-by-drop to wells with a gentle swirl of the plate to mix (1 ml final volume).
- Incubate plates as usual for 24 - 72 hours.

plate	Well surface area	Media (vol/well)	Transfection mixture volume	Fresh media volume	siRNA transfection	
					siRNA (20 nM)	Nanocin™-RNAi
24-well	2 cm^2	500 µl	25 µl	475 µl	10 pmol	2 µl
12-well	4 cm^2	1 ml	50 µl	950 µl	20 pmol	4 µl
6-well	10 cm^2	2.5 ml	125 µl	2375 µl	50 pmol	10 µl
60-mm	20 cm^2	5 ml	250 µl	4750 µl	100 pmol	20 µl

Notes:

- growth media may contain 10% FCS and antibiotics
- when using lower siRNA concentrations, reduce Nanocin™-RNAi volume proportionately
- to optimize siRNA Transfection: vary cell number, DNA and Nanocin™-RNAi concentrations. See table for suggestions on plate set-up. The amounts of Nanocin™-RNAi and DNA used can be varied +/- 50% to optimize.