

### **RNA transfection protocol**

### FOR TECREA PRODUCTS TNR-250, TNR-500 AND TNR-1000

#### **Product information**

Nanocin RNA is an innovative transfection reagent dedicated to efficient and non-toxic products for research, but also highly compatible with clinical development, meaning you can carry research from lab to clinic with confidence. Nanocin RNA allows the 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 RNA** is tested using biophysical methods and by ensuring efficient delivery of siRNAs into HEK293T cells, assessed by qRT-PCR.

#### 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 RNA 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-rna/

#### Technical resources and scientific advice

Tecrea provides extensive technical support and scientific guidance for your experiments involving **Nanocin** products. Please contact us for more information. info@tecrea.com / Frequently asked questions.

#### **Helpful information**

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

**Nanocin RNA** products are effectively non-toxic, meaning they can facilitate multiple transfections.

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

#### **Contents and ordering**

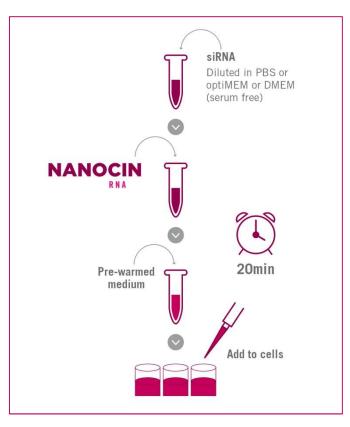
Unit size (mL)	Transfections*	Cat No.	
0.25	50-75	TNR-250	
0.5	100-150	TNR-500	
1.0	200-300	TNR-1000	

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

#### **Related products**

Product Nanocin PLASMID	<b>Cat No.</b> TNP-250, TNP-500, TNP-1000, TNP-10000
Nanocin PRO (for protein & peptide delivery)	TNPRO-250, TNPRO-500
Nanocin SM (for small molecule delivery)	TNSM-250, TNSM-500

#### **Protocol overview**



## Standard RNA TRANSFECTION PROTOCOL

# Rapid RNA 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.

Use this rapid protocol to transfect 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

- Seed and grow cells to 60-80% confluences.
- Vortex Nanocin RNA reagent for 10seconds and centrifuge briefly.

#### Set-up

• Vortex **Nanocin RNA** reagent for 10 seconds and centrifuge briefly.

#### **START Transfection**

Step 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 by pipetting the full volume up and down 5-10 times. Add 4 μl of Nanocin RNA reagent and mix thoroughly, pipetting the full volume up and down 5-10 times. Incubate for 20 minutes at room temperature.

**Step 2. Transfect:** Transfer tubes from ice to rack at room temperature. Add 950  $\mu$ l of pre-warmed growth medium to each tube prepared in step 1 so each tube totals 1000  $\mu$ l in volume, then 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.

#### **START Transfection**

Step 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  $\mu$ l. Mix thoroughly by pipetting the full volume up and down 5-10 times. Add 4  $\mu$ l of Nanocin RNA reagent and mix thoroughly, pipetting 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  $500~\mu l$  to each well (one half of final volume in well).

**Step 2. Transfect:** Add 450  $\mu$ l of pre-warmed growth medium to each tube prepared in step 1 so each tube totals 500  $\mu$ l in volume. Mix thoroughly, by pipetting the full volume up and down 5-10 times. Add drop-by-drop to wells with a gentle swirl of the plate to mix, totalling 1 ml final volume. Incubate plates as usual for 24-72 hours.

#### Alternative volumes for other plates' formats

Plate	Well surface area	Media (Vol/Well)	Transfection mix volume	Fresh media volume	siRNA transfection	
					siRNA (20nM)	Nanocin RNA
24-well	2 cm <sup>2</sup>	500 μΙ	25 μΙ	475 μΙ	10 pmol	2 μΙ
12-well	4 cm <sup>2</sup>	1 ml	50 μΙ	950 μΙ	20 pmol	4 μΙ
6-well	$10\mathrm{cm}^2$	2.5 ml	125 μΙ	2375 μΙ	50 pmol	10 μΙ
60-mm	$20\mathrm{cm}^2$	5 ml	250 μΙ	4750 μΙ	100 pmol	20 μΙ

#### Notes

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

