Nanocin™

Protocol for HEK293T cells

plasmid TRANSFECTION PROTOCOL for Tecrea Ltd products:

- THFP-250
- THFP-500
- THFP-1000

Transfection and Cell Delivery
From lab to clinic

Product information

Nanocin™ 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™ 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™ 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

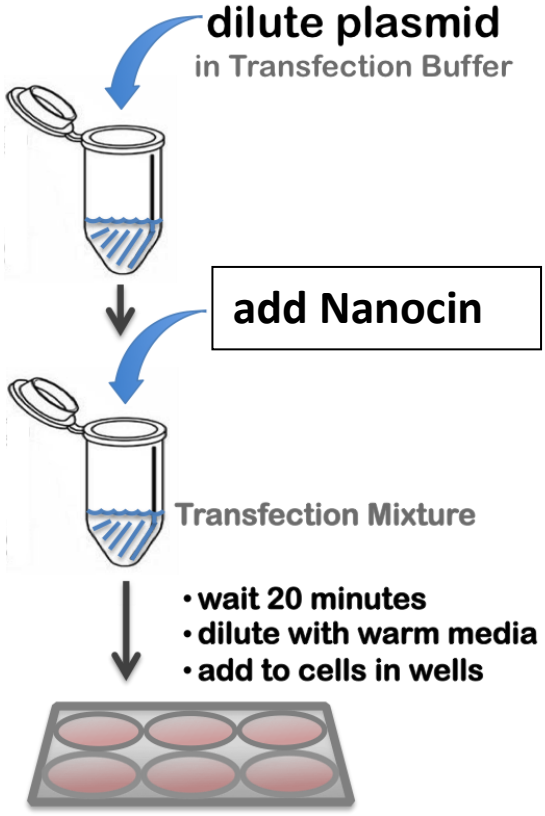
FAQs at: www.tecrea.co.uk/suport/FAQs
Troubleshooting guide: www.tecrea.co.uk/support

☺ **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™ products have such low toxicity that experiments can involve multiple, serial transfections

TOP TIP #3 Nanocin™ 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.

PROTOCOL OVERVIEW



see next page for details

STANDARD

PLASMID TRANSFECTION PROTOCOL applied to HEK293T cells

Use this protocol to transfect plasmid DNA into **HEK293T** 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

- Day 0: Seed a 12-well plate with a cell density of **2.28 x 10^5 cells/ml** with **1ml** of cell suspension per well. Incubate for 24 hours.
- Day 1: Vortex Nanocin™ reagent for 10 seconds and centrifuge briefly..

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].

Add 2.5 µl of Nanocin reagent (50 µl total volume), mix thoroughly (pipette the full volume up and down 5-10 times). Incubate for 20 minutes at room temperature.

2. Transfect:

Add 950 µl 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 onto well walls, with a gentle swirl of the plate to mix. Incubate plates as usual for 48 to 72 hours.

(see plate and media recommendations below)

RAPID

PLASMID TRANSFECTION PROTOCOL applied to HEK293T cells

Use this *rapid* protocol to transfect plasmid into **HEK293T** 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

- Day 1: Seed a 12-well plate with a cell density of **4.56 x 10^5 cells/ml** with **1ml** of cell suspension per well. Incubate for 1-2 hours.
- Vortex Nanocin™ reagent for 10 seconds and centrifuge briefly.

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].

Add 2.5 µl of Nanocin reagent (50 µl total volume), mix thoroughly (pipette the full volume up and down 5-10 times). Incubate for 20 minutes at room temperature.

2. Transfect:

Add 950 µl pre-warmed growth medium to each tube prepared in step 1 (1000 µl total volume), mix thoroughly and then add drop-by-drop to cells in the well - gently swirl the plate to mix. Incubate plates as usual for 48 to 72 hours.

(see plate and media recommendations below)

Plate	Confluence	Well surface area	Media (vol/well)	Transfection mixture volume	Fresh media volume	Plasmid transfection	
						pDNA	Nanocin
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:

- use transfection mixture within 60 minutes after preparation
- mix thoroughly at all mixing steps by pipetting up & down the full volume 5-10 times
- cells are incubated at 37°C, 5% CO_2 and 95% air

Tecrea Ltd, 2 Royal College St., London, NW1 0NH
www.tecrea.co.uk
info@tecrea.co.uk

Additional recommendations

for HEK293T culture and transfection

Cells supplier

ATCC : HEK293T Human Embryonic Kidney cells, ATCC® CRL 3216™

Recommended culture flask and 12-well plate

Cell Bind T75 flask (Corning catalog number 3290) and **CellBind 12-well plate** (Corning catalog number 3336)

Recommended growth and transfection medium

FreeStyle 293 Expression Medium (Life Technologies Catalog number: 12338-018)

HEK293T culture in FreeStyle medium

Split cells every 3-4 days (when they reach approximately 80% confluency)

- Remove the cells from the surface by giving couple of taps on the sides of the flask. A detachment reagent is not required
- Transfer the suspension in a 50ml tube
- Centrifuge at 200g for 10min
- Discard the medium by using a pipette and resuspend the pellet in 10ml FreeStyle Medium
- Perform cell counting and determine the viable cell count
- Prepare a cell suspension at the required cell density, seed the plate or the flask and incubate as usual

Important notes :

- The HEK293T cells are quite sensitive and request gentle manipulation especially during the transfection step. The recommended medium, culture flask and 12-well plate are the best material to have a good adherence and an efficient transfection
- Cells should be “adapted” to growth in a serum free medium. It is advised to revive the HEK293T cells in a complete medium (for example DMEM + FBS). The first split after revival should be done in complete DMEM; after that, all splits can be done in FreeStyle medium only.
- It is recommended to do at least 3 or 4 splits (in FreeStyle medium) before performing transfection.

Tecrea Ltd, 2 Royal College St., London, NW1 0NH
www.tecrea.co.uk
info@tecrea.co.uk