

BIOMEDICAL RESEARCH SERVICE CENTER

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NANO DNA Transfection Kit (Cat #: N-104)

Components: NANO Solution A: 3 ml, store at 4°C (100 wells of a 24-well plate)
NANO Solution B: 3 ml, store at 4°C
NANO Solution C: 30 ml, store at 4°C

PRODUCT DESCRIPTION: Our NANO DNA Transfection Kit is based on incorporation of DNA into uniquely formulated nanoparticles in the presence of a polymer. The nanoparticle formulation allows researchers to obtain high DNA transfection efficiency. Each cell type should be optimized by researchers to assess the optimal transfection time, potential reagent toxicity and transfection efficiency. The kit is stable for at least one year if handled and stored properly.

PROTOCOL: The protocol is written for DNA transfection in wells of a 24-well plate, and can be scaled up proportionately for larger culture vessels. It can be modified for suspension cultures. Use sterile techniques throughout the protocol.

Adherent cells plated on a 24-well plate:

1. Each well requires 30 μ l NANO Solution A, 30 μ l NANO Solution B, 0.3 ml NANO Solution C, and 0.6 ml culture medium (determined by end user). First calculate the volume of NANO Solution C and culture medium required for each transfection experiment. Warm a sufficient amount of NANO Solution C and culture medium (each in a loose cap tube) in a CO₂ incubator for at least 10 min prior to transfection. This step ensures the correct temperature and pH for transfection.
2. Add 30 μ l NANO Solution A to a 0.5-ml microtube. Then add 0.5 – 1 μ g of a plasmid DNA to NANO Solution A. The tube is now referred to as the DNA tube.
3. Use a fine tip to add 30 μ l NANO Solution B drop by drop to the DNA tube while vortexing the DNA tube at the same time. Vortex the DNA tube for another 10 sec after addition. Keep the DNA tube at room temperature for 20 min.
4. Remove cells (plated in a 24-well plate) and NANO Solution C from the CO₂ incubator. Aspirate off culture medium from each well. Add 0.3 ml NANO Solution C to each well.
5. Transfer DNA solution (60 μ l) into NANO Solution C above the monolayer, and agitate plate reciprocally several times to mix. Incubate cells in a humidified CO₂ incubator at 37°C for 6 – 8 hours.
6. Remove plate and prewarmed culture medium from CO₂ incubator. Tap plate a few times to loosen nano particles attached to cells. Aspirate off solution from each well. Rinse cells with 0.3 ml culture medium per well, tap plate a few times, and aspirate culture medium.
7. Feed cells with 0.3 ml culture medium per well. Incubate cells in a CO₂ incubator for 2 – 3 days before assaying for gene expression.

Suspension cells plated on a 24-well plate:

1. Perform steps 1 – 3 as described above for adherent cells.
2. Spin down cells and gently resuspend the cell pellet in 0.3 ml NANO Solution C.
3. Transfer DNA solution (60 μ l) into cell suspension and agitate plate to mix contents. Incubate cells in a CO₂ incubator at 37°C for 6 – 8 hours.
4. Spin down cells, aspirate off medium, and resuspend cells in 0.3 ml prewarmed culture medium. Spin down cells again and aspirate off medium. Finally resuspend cells in 0.3 ml prewarmed culture medium.
5. Incubate cells in a CO₂ incubator for 2 – 3 days before assaying for gene expression.