

Transient transfection of Jurkat T cells 24-well plate format

- Subculture the cells one day before transfection

 1. Dilute 33ng of PMA en 250ng of plasmid DNA in 47 μ L sterile H₂O
 2. Add 3 μ L SAINT-18 and incubate for 1-2min (RT) and pipette in a 24-well
 3. Count the jurkat cells and centrifuge at max. 350xg for 10minutes
 4. Resuspend the pellet to 4x10⁵/mL in serum-free medium
 5. Add 250 μ L cell suspension to the well and incubate for max. 15 minutes
 6. Add minimal 500 μ L prewarmed complete medium
 7. Incubate at 37°C and 5% CO₂ until use

Additional notes:

- a. *Prior to transfection, cells should be rigorously maintained at 0.5-1.0 x 10⁶ cells/mL for 2-3 days, as density will affect both transfection efficiency as well as cell viability.*
- b. *The amount of plasmid DNA used per transfection will vary depending on the level of expression desired. However, every transfection should contain approximately 200-400ng DNA.*
- c. *The amount of SAINT-18 can be varied to increase efficiency from 2-5 μ L*
- d. *After transfection, the cells should be incubated the first 10-15 minutes in serum-free medium to obtain a maximum efficiency.*
- e. *Do not exceed this incubation period for more than 15 minutes as this leads to a decrease in cell viability.*