

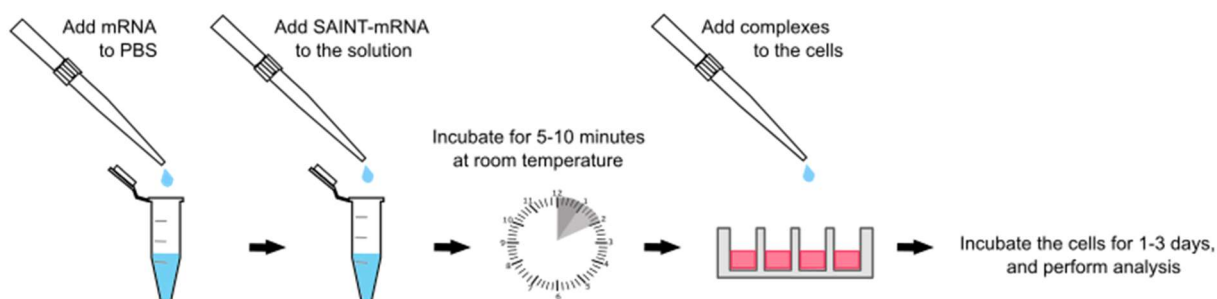
SAINT-MRNA

MESSENGER RNA DELIVERY SYSTEM



This protocol is provided for transfections using SAINT-mRNA (Cat. No. SM-2005-01, SM-2005-02, SM-2005-04). The amounts in this protocol are for a single well of a 24-well plate. Please see Table 1 at the bottom of this page for other formats. This protocol is suitable for many cell types, however for optimal results further optimization for your cell type of choice is possible.

General transfection protocol:



1. Prepare cells for transfection.
 - **Adherent cells:** one day prior to the transfection, plate cells in 0.5 ml growth medium so that the cells will be 50-80% confluent at the time of transfection.
 - **Suspension cells:** on the day of transfection, plate cells in 0.5 ml of growth medium so that the cells will have reached maximum density when transgene expression is tested.
2. Allow the vial of SAINT-mRNA to reach room temperature.
3. Vortex the SAINT-mRNA thoroughly, for approximately 30 seconds.
4. Prepare complexes using a mRNA (μg) to SAINT-mRNA (μl) ratio of 1:20.
5. For each transfection sample, prepare complexes as follows:
 - a. Dilute 0.25 μg mRNA in 50 μl PBS.
 - b. Add 5 μl SAINT-mRNA into the mRNA/PBS solution and resuspend gently.
6. Incubate the mixture for 5-10 minutes at room temperature (solution may appear cloudy).
7. Add the complexes directly to the wells containing the cells.
8. Incubate the cells at 37°C in a CO₂ incubator for 1-3 days prior to testing for transgene expression.

Table 1. Recommended amounts per well for commonly used multi-well plates

Format	Growth medium (μl)	mRNA (ng)	PBS (μl)	SAINT-mRNA (μl)
96-well	100-200	50	10	1
48-well	200-350	125	25	2.5
24-well	400-750	250	50	5
12-well	750-1500	500	100	10
6-well	2000-3000	1000	200	20

Note: The SAINT-mRNA reagent is stable for at least 1 year at 4°C. Do not freeze!