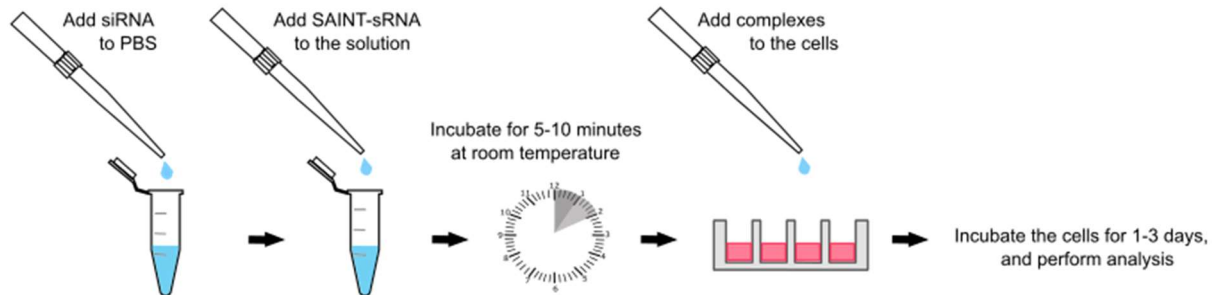


This protocol is provided for transfections in serum-free culture medium using SAINT-sRNA (Cat. No. SR-2003-01, SR-2003-02, SR-2003-04). The amounts in this protocol are for a single well of a 24-well plate. Please see Table 1 at the bottom of this page for other formats. The general transfection protocol in serum-containing growth medium can be found at our website: www.synvoluxproducts.com.

Serum-free transfection protocol:



1. Prepare cells for transfection.
 - **Adherent cells:** one day prior to the transfection, plate cells in 0.5 ml growth medium with serum so that the cells will be 50-80% confluent at the time of transfection.
 - **Suspension cells:** Just prior to preparing the complexes, suspend $0.5-1.5 \times 10^6$ cells in 0.5 ml of serum-free growth medium.
2. Allow the vial of SAINT-sRNA to reach room temperature.
3. Vortex the SAINT-sRNA thoroughly, for approximately 30 seconds.
4. Prepare complexes using a siRNA (μg) to SAINT-sRNA (μl) ratio of 1:40.
5. For each transfection sample, prepare complexes as follows:
 - a. Dilute 0.125 μg siRNA in 50 μl PBS.
 - b. Add 5 μl SAINT-sRNA into the siRNA/PBS solution and resuspend gently.
7. Incubate the mixture for 5-10 minutes at room temperature (solution may appear cloudy).
8. Add complexes to cells:
 - **Adherent cells:** Aspirate growth medium from the cells and fill up the complexes to 0.5 ml with serum-free growth medium. Add the complexes (0.5 ml) to the cells.
 - **Suspension cells:** Add the prepared complexes dropwise to the well.
9. Incubate cells during 2-3 hours at 37°C in a CO₂ incubator.
10. Add 1 ml growth medium with serum to the wells and incubate the cells at 37°C in a CO₂ incubator for 1-3 days prior to testing for gene knockdown.

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

Format	Growth medium (μl)	siRNA (ng)	PBS (μl)	SAINT-sRNA (μl)
96-well	100-200	25	10	1
48-well	250-350	62.5	25	2.5
24-well	400-750	125	50	5
12-well	750-1500	250	100	10
6-well	2000-3000	500	200	20

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