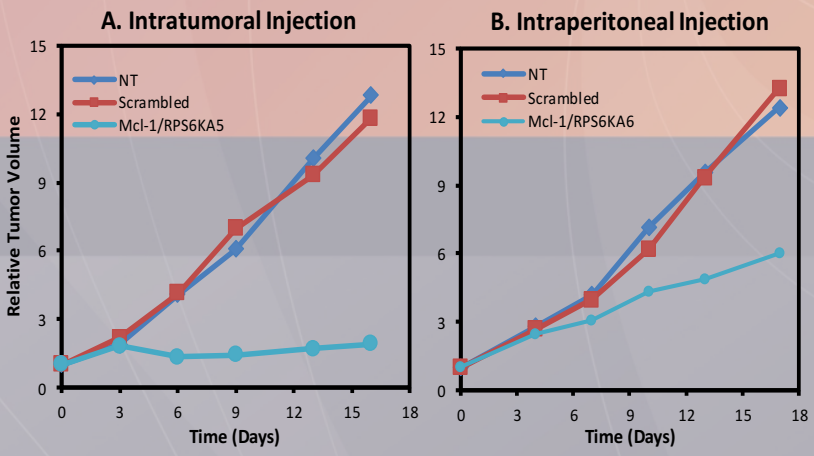
tailored transfection reagent: In Vivo RNA-Fect

PRODUCT NUMBERS: 70-10 and 70-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: 4 °C

Product Description

In Vivo RNA-Fect is a highly effective transfection reagent for use in animal models. **In Vivo RNA-Fect** is a synthetic amphiphilic polymer that is specifically tailored for siRNA delivery in animal models. It is capable of undergoing multivalent interactions with siRNA and encapsulating co-incubated siRNA molecules into ~100 nm particles with a net positive charge. The complexation between the siRNA and **In Vivo RNA-Fect** occurs in aqueous buffers, obviating the need for organic solvents during preparation. **In Vivo RNA-Fect** is a non-integrating carrier of siRNA, so that the genetic make-up of host cells is not altered after treatment with the transfection reagent. **In Vivo RNA-Fect** has been tested and found effective after administration via systemic routes, such as intravenous and intraperitoneal. As with all transfection reagents and delivery agents, formulation of **In Vivo RNA-Fect** with siRNA may need to be optimized for specific applications.

Tumor growth profiles as a result of siRNA delivery with In Vivo RNA-Fect. (Left) Subcutaneous MDA-MB-231 tumors were established in nu/nu mouse. siRNAs (a control and a therapeutic siRNA) formulated with **In Vivo RNA-Fect** were injected (x3) intravenously and tumor growth was assessed over a period of 16 days. **(Right)** Subcutaneous tumors from MDA-MB-231 cells were established in nu/nu mouse. siRNAs (a control and a therapeutic siRNA) formulated with **In Vivo RNA-Fect** were injected (x3) intraperitoneally and tumor growth was assessed over a period of 16 days.



| Benefits of In Vivo RNA-Fect

- High transfection efficiency in primary cells.
- Simple protocol. No need to stabilize particles for injection into animal models.
- Minimal toxicity compared to other commercial reagents, leading to efficacious performance without altering normal physiological state.
- Non-integrating transfection reagent eliminates adverse effects due to host genome integration.

| Considerations for Transfection Protocol

The following procedure is recommended for preparation of siRNA particles with **In Vivo RNA-Fect** and subsequent delivery in animal models of cancer therapy. Please ensure all reagents are at the room temperature before use.

- We recommend to employ tumors of 50-100 mm^3 volume at the study onset. The tumors should be established in desired animal models according to the growth of individual types of cells.

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- Recommended ratio of siRNA and **In Vivo RNA-Fect** in complex formation is 1:8 to 1:12 (w/w) ratio. A ~20% excess volume of complexes should be prepared to account for handling losses.
- The final recommended amount of siRNA to be injected is expected to vary depending on the response of target cells. An siRNA dose of 1 to 10 µg siRNA (per 20 g mouse) is recommended range to use.
- We recommend to prepare the complexes in endotoxin-free, sterile saline (150 mM NaCl) for injection.
- Injection volume for specific animal models should be consulted with a veterinarian; as a starting point, 100 µL injection volume is recommended for intravenous injections, while 300 µL is recommended for intraperitoneal injections in a typical 20g mouse model.
- Typical volumes to be used in preparing complexes are indicated below, based on the dose of siRNA desired.

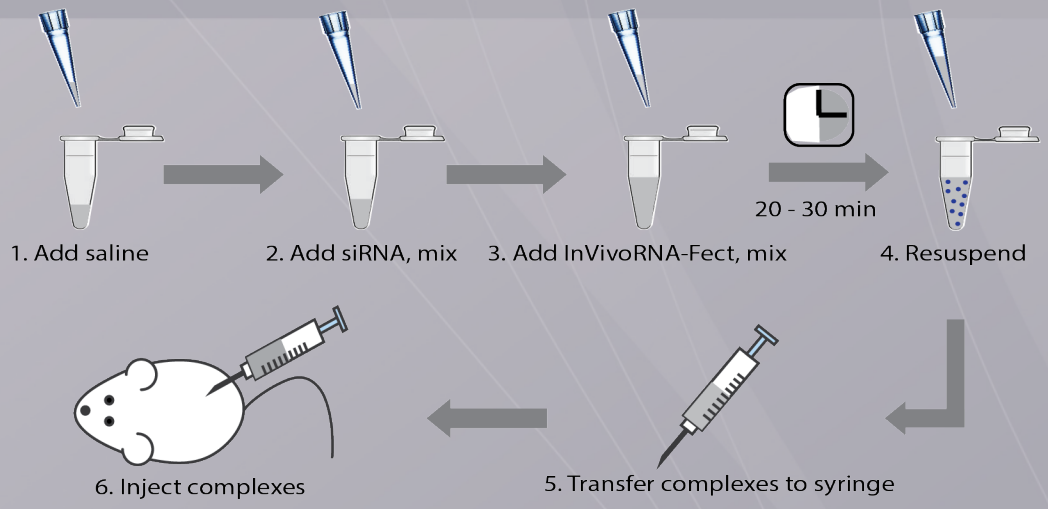
siRNA Dose	Saline (µL)	siRNA volume*	In Vivo RNA-Fect*	Injection Medium
1 µg	316.3 µL	2.75 µL	11 µL	300 µL
3 µg	289.7 µL	8.25 µL	32 µL	300 µL
5 µg	261.2 µL	13.75 µL	55 µL	300 µL

- Recommended volumes based on 0.4 mg/mL siRNA and 1 mg/mL **In Vivo RNA-Fect** solutions.

| Procedure

1. Add the desired volume of saline to sterile 1.5 mL plastic (microcentrifuge) tubes.
2. Add appropriate volume of siRNA solution to saline in tubes and vortex gently for 3 sec.
3. Add undiluted **In Vivo RNA-Fect** solution to the siRNA solution in saline. Vortex gently for 3 sec and incubate for 20 min at room temperature.
4. Re-suspend the siRNA complexes in solution using a pipette at the end of incubation period.
5. Withdraw complexes into a suitable size syringe (e.g., 28G) and inject intravenously or intraperitoneally depending on the desired administration route.
6. Observe and return the animals to the housing unit as per approved protocol.

| Graphical Procedure



| References

- Aliabadi et al., J. Controlled Release (2013) 172: 219-228.
- Parmar et al., Frontiers Bioeng. Biotechnol. (2015) 3:14.