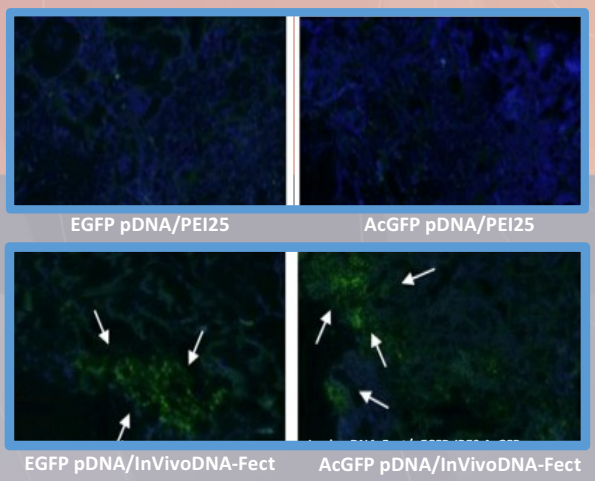
tailored transfection reagent: InVivoDNA-Fect

PRODUCT NUMBERS: 60-10 and 60-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: 4 °C

Product Description

InVivoDNA-Fect is a highly effective delivery agent for use in animal studies. **InVivoDNA-Fect** is a synthetic amphiphilic polymer that is specifically tailored for plasmid DNA delivery. It is capable of undergoing multivalent interactions with DNA and encapsulating co-incubated DNA molecules into 100-150 nm particles with a net positive charge. The complexation between the plasmid DNA and **InVivoDNA-Fect** occurs in aqueous buffers, obviating the need for organic solvents during preparation. **InVivoDNA-Fect** is a non-integrating carrier of DNA, so that the genetic make-up of host cells is not altered after treatment with the delivery reagent. **InVivoDNA-Fect** has been tested and found effective in different types of attachment dependent cells as well for cell culture use, but users are advised to test the efficacy of the reagent with their particular cell type to confirm utility in specific applications. As with all delivery agents, formulations of **InVivoDNA-Fect** with plasmid DNA may need to be optimized for specific applications *in vivo*.



Delivery of plasmid DNA using InVivoDNA-Fect and high molecular weight PEI. Two different GFP expression plasmids (EGFP and AcGFP) were used for subcutaneous implantation in rats with collagen implants. The delivery agents were either 25 kDa branched PEI **(top 2 pictures)** or **InVivoDNA-Fect (bottom two pictures)**. The implants were recovered and visualized for GFP expression by epifluoresecent microscopy. GFP positive cells were primarily observed when **InVivoDNA-Fect** was used for plasmid delivery (see arrows).

| Benefits of InVivoDNA-Fect

- Can be administered with simple buffers such as saline.
- Also functional with broad range of cells under cell culture conditions.
- Minimal toxicity compared to other commercial reagents, leading to sustained transfections in animals.
- Non-integrating delivery agent eliminates adverse effects due to possible genotoxicity.

| Transfection Protocol

The following procedure is recommended for use of plasmid DNA particles with **InVivoDNA-Fect** in animals. Please ensure all reagents are sterile and at room temperature for the procedures.

- All ethics approvals should be secured before the use of **InVivoDNA-Fect**. Animals should be acclimated to the surgical conditions and sterile procedures should be followed for administration of **InVivoDNA-Fect**.
- Recommended amounts of plasmid DNA and **InVivoDNA-Fect** reagent are shown in the Table for typical formulations. The recommended amounts of plasmid DNA and **InVivoDNA-Fect** are 5-10 µg for implantation and 10-20 µg for systemic injection, with DNA: **InVivoDNA-Fect** ratios of 1:5 and 1:10.

- We recommend DNA and **InVivoDNA-Fect** concentrations to be optimized for each application. Suggested ranges for optimization are 1 to 20 µg for plasmid DNA and 5 to 200 µg for **InVivoDNA-Fect**. The amounts shown below are for a single administration, assuming 0.4 mg/mL DNA and 2 mg/mL **InVivoDNA-Fect** stock solutions. The calculations are intended for a 200 g animal (typical of a rat) and administered doses/volumes could be adjusted depending on weight of animals and the number of replicates in a study group.
- We recommend preparation of 10% excess volume to account for any possible loss due to pipetting.
- Sterile saline is recommended for complex preparation but cell culture medium (without antibiotics and serum) could be also used.

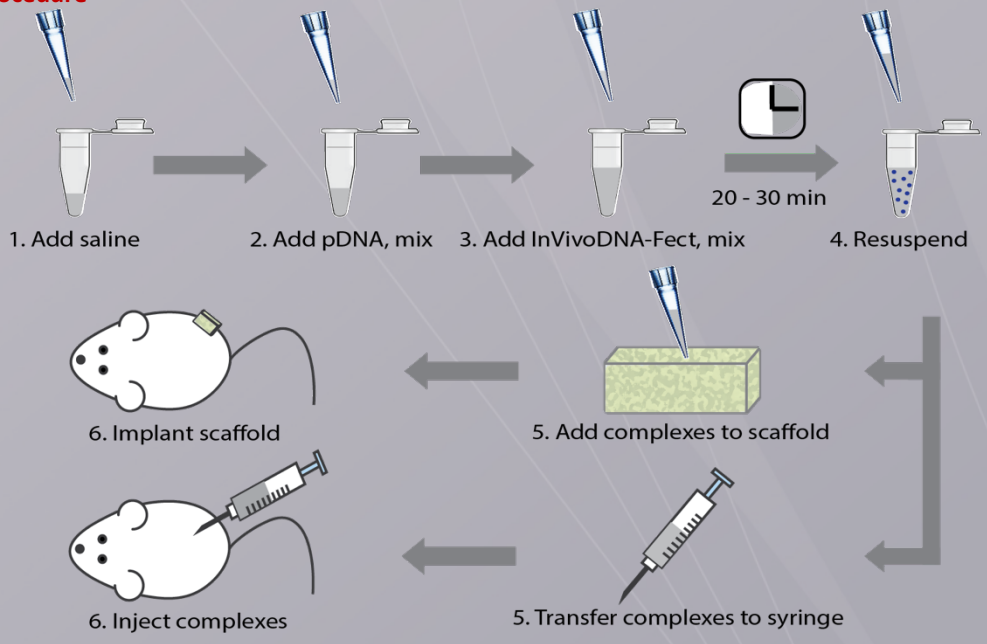
Implant Dose	pDNA:InVivoDNA-Fect Ratio	Plasmid DNA (µL)*	InVivoDNA-Fect (µL)*	Volume of Saline (µL)
10 µg	5	25	25	250
20 µg	5	50	50	200
10 µg	10	25	50	225
20 µg	10	50	100	150

- Recommended volumes based on 0.4 mg/mL plasmid DNA and 2 mg/mL **InVivoDNA-Fect** solutions.

| Procedure

1. Add the desired volume of saline to sterile 1.5 mL plastic (microcentrifuge) tubes.
2. Add appropriate volume of DNA solution to saline in tubes and vortex gently for 3 sec.
3. Add undiluted **InVivoDNA-Fect** solution to DNA solution. Vortex gently for 3 sec and incubate for 20 min at room temperature.
4. Re-suspend the DNA complexes in solution using a pipette at the end of incubation.
5. If the complexes are to be injected directly, withdraw the complexes into a suitable size syringe and administer to animals directly (as per pre-approved ethics protocol).
6. If the complexes are to be implanted with a biomaterial scaffold, soak the biomaterial with complexes using a pipette and implant at the appropriate anatomical site (as per pre-approved ethics protocol).

| Graphical Procedure



| References

- Rose et al., Biomaterials Science (2014) 2: 833-842.
- Rose et al., Biomaterials (2012) 33: 3363-3374.