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RiboCraft Alpha (*vivo*)

in vivo mRNA transfection quick reference protocol

❑ Product introduction:

RiboCraft Alpha stands out as a versatile transfection reagent facilitating efficient mRNA delivery in both *in vitro* and *in vivo* settings. Following intravenous (IV) injections, it predominantly targets the spleen.

❑ Materials needed:

- RiboCraft Alpha (*vivo*) transfection reagent (Cat# AAN1L10006, store at 2 – 8 °C)
- mRNA stock solution (1 mg/mL)
- RNase-Free water
- RNase-Free 10X PBS buffer

❑ General guidance:

The recommended doses for mRNA delivery in mice are given in the table below. The mRNA dose and the volume of RiboCraft Alpha (*vivo*) should be further optimized according to the transfection assay and animal model.

Parameter	Administration route	mRNA (μg/mouse)	RiboCraft Alpha (*vivo*) (μL/mouse)	Final mRNA concentration in the formulation (μg/μL)	Final injection volume (μL/well)
Recommended quantities to start with	IV	5	12	0.05	100
Optimization range	IV	5 – 30	6 – 50	≤ 0.15	≤ 200

- $V(H_2O)$ = V(Formulation) – V(mRNA) – V(RiboCraft Alpha (*vivo*)) – V(10X PBS)
- Add 10X PBS to adjust the injection solution to be a 1X PBS solution.
- For example, to prepare a master solution for 4 mice, dilute 20.0 μL mRNA (1 mg/mL) with 292.0 μL RNase-free H_2O, followed by the addition of 48.0 μL RiboCraft Alpha (*vivo*). Mix thoroughly in each step and allow the solution to incubate at room temperature for 5 minutes. Add 40.0 μL 10X PBS to make a total of 400 μL 1X PBS solution for injection. Mix the solution gently using a pipette.
- Adjust the ratio of RiboCraft Alpha (*vivo*) to mRNA within the recommended range for optimal results. At higher mRNA doses, a smaller amount of RiboCraft Alpha (*vivo*) can be used to conserve reagents while ensuring both efficacy and safety.

❑ Transfection

Dilute mRNA in RNase-free H_2O. Mix well by pipetting or vortex. Spin down briefly.

Add RiboCraft Alpha (*vivo*). Mix well by pipetting (~30 times). Spin down briefly. Incubate 5 minutes at room temperature.
(The nanocomplexes are formed via rapid self-assembly and are stable at room temperature up to 30 minutes.)

Add 10X PBS to obtain a final 1X PBS solution for injection. Mix the solution gently with a pipette. Nanocomplexes are stable in PBS at RT up to 30 minutes.

Perform IV injections into animals. Measure transfection 3 to 48 hours post injection.

❑ Tips

- Ensure all reagents are RNase-free.
- Store RiboCraft Alpha (*vivo*) properly at 2 – 8 °C, or at -20 °C for extended storage if not used within a short period.
- Using chemically modified mRNA (e.g. 5-methylcytidine, pseudouridine) could improve transfection efficiency.
- Use a common reporter gene mRNA (e.g. Fluc, eGFP mRNA) as a positive control.
- Adjust the mRNA dose and the volume of RiboCraft Alpha (*vivo*) to improve transfection performance and decrease toxicity.
- Measure transfection at earlier time points (e.g. 3, 6, 24 h).