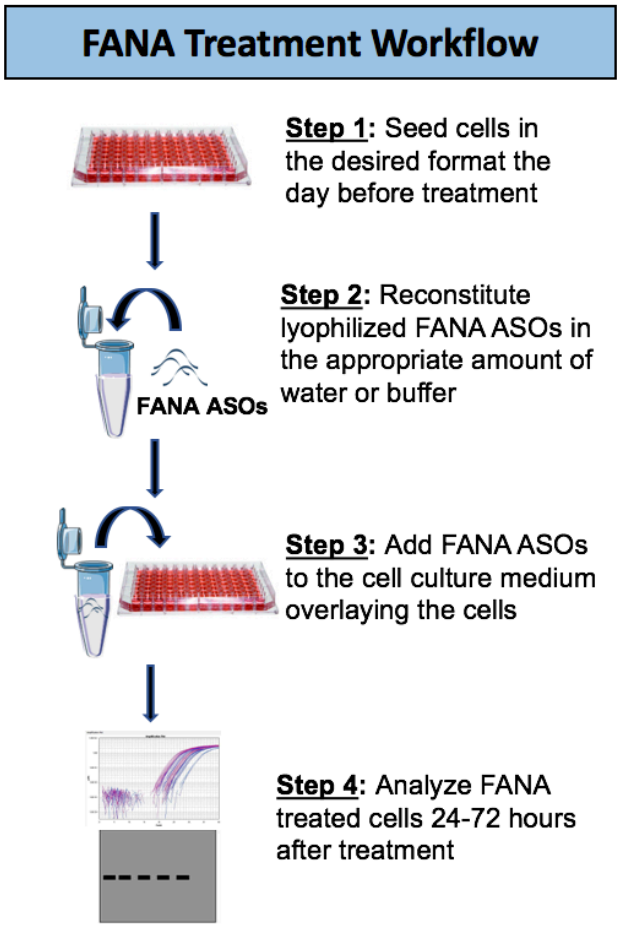
Gymnotic Delivery Protocol: FANA Antisense Oligonucleotides (FANA ASOs) for mRNA & lncRNA knockdown

Lipid-based transfection reagents and electroporation systems are widely utilized, conventional methods to deliver siRNA and other conventional oligonucleotides into the cells. However, in many primary cells, particularly immune cells, hematopoietic cells and neurons, lipid reagents and electroporation are associated with high toxicity and poor transfection efficiency. Alternative delivery methods, such as viral vectors, require laborious optimization and viral production steps, and carry associated risk of genome integration.

FANA Antisense Oligonucleotides (FANA ASOs) have unique, state-of-the-art chemical modifications that allow for highly efficient cell delivery in the absence of transfection reagents, thus eliminating cell toxicity associated with lipid transfection reagents and electroporation. FANA oligos have effective delivery and superior performance and work in a wide range of mammalian cell types, conventional cell lines, difficult-to-transfect primary cells (B-cells, T-cells, neurons, etc.), as well as *in vivo* study models.



FANA Delivery in Mammalian Cells: Protocol

Note: The following is a general protocol for the use of FANA in mammalian cells. It can be adapted for different cell types and different culture vessel formats.

1. Plate cells in their optimum growth medium and in the desired well or culture plate format.

- Plate cells the day before (for adherent cells) or prior to treatment with FANAs (for suspension cells) in complete media at a 30% - 50% cell density (or at densities optimized for growth conditions and the end-point of the assay). In case of adherent cells, allow the cells to adhere.

2. Prepare FANA stock solution by reconstituting lyophilized FANA ASOs at the desired concentration. If the stock solution has already been prepared, skip to step 3.

- Resuspend lyophilized FANA ASOs using the appropriate volume of sterile water or buffer. Pipette solution up and down 3-5 times while avoiding introduction of bubbles. Let the vial sit at room temperature for 5-10 minutes. Centrifuge for 30-45 seconds to collect solution to bottom of the tube. It is recommended to make several aliquots fo the stock solution to avoid multiple freeze thaw cycles.

3. **Add FANAs to the cells and media to the desired final concentration.**

   For silencing experiments, the working concentration of FANA ASOs can vary from **500 nM to 5 µM**. It is highly recommended to perform a dose response using 2-3 working concentrations **(500 nM, 2.5 µM and 5 µM)** to determine the most optimum concentration for your application. In some specific cases, higher concentrations may be required.

   - For adherent cells: aspirate the growth media and overlay cells with media containing FANAs or add FANAs stock directly to the media overlaying the cells. Mix gently.
   - For suspension cells, pellet the cells by low-speed centrifugation and gently resuspend the cell pellet in media containing FANAs. Alternatively, add FANAs ASOs directly to the media overlaying the cells.

4. **Analyze FANA treated cells after 24 – 72 hours post treatment.**
   - **Note:** Uptake of fluorescently-labeled FANA can be observed as early as 4-8 hr, but full knockdown is best assessed at 24-72 hours post treatment.

Reference calculations: Amount of FANA ASOs

Cell culture plate	96-well	24-well	12-well	6-well
FANA ASO[1] stock (µL)	1 µL	5 µL	10 µL	30 µL
FANA ASO[1] used (moles)	100 pmole	500 pmole	1 nmole	3 nmole
Cell culture media (µL)	100 µL	500 µL	1000 µL	3000 µL
Cell number (per well)[2]	$0.5x10^5$	$2.5x10^5$	$0.5x10^6$	$1x10^6$

[1] The amount of FANA shown yields a final concentration of 1 µM using 100 uM stock.

[2] The optimal seeding cell density will vary with the cell type, cell size, growth characteristics and the end-point of the assay. For this table, HeLa cells at 50% confluency were used at the time of FANA treatment. In general, a confluency of 30 – 50% is recommended at the time of FANA ASO treatment.

Additional Notes:

(1) Depending upon the experiment different time points can be used to measure knock down or related effects for up to several days (and weeks in some cases) using a single dose.

(2) In certain cases (especially for very fast-growing cells) if the knock down effect may be reduced after a few days. In such cases, simply add more FANA ASOs to the cell culture to maintain knockdown.

(3) FANA oligos can be fluorescently labeled (or with any desired label) to monitor cellular uptake.

Storage: (1) FANA ASOs are shipped in lyophilized form. Upon arrival, store them in -20°C. (2) When ready to use, resuspend FANA ASOs in sterile water or appropriate buffer at the desired concentration. Aliquot resuspended FANA ASOs in aliquots to avoid multiple freeze-thaw cycles.

Contact and customer support: For questions on products, protocols or new orders please reach out to us **customercare@aumbiotech.com** or visit us at **www.aumbiotech.com**.