Gymnotic Delivery Protocol (No transfection reagents needed)

AUM*silence* Antisense Oligonucleotides (AUM*silence* ASOs)

Application: mRNA & lncRNA knockdown or miRNA inhibition

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.

AUM*silence* Antisense Oligonucleotides (AUM*silence* ASOs) are uniquely designed and manufactured using 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. AUM*silence* 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.

Protocol: AUM*silence* ASO Delivery in Mammalian Cells.

Note: The following is a general protocol for the use of AUM*silence* ASOs 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 AUM*silence* ASOs (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.

   Prepare AUM*silence* ASO stock solution by reconstituting lyophilized AUM*silence* ASOs at the desired concentration. If the stock solution has already been prepared, skip to step 3.
   - Resuspend lyophilized AUM*silence* 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 of the stock solution to avoid multiple freeze thaw cycles.

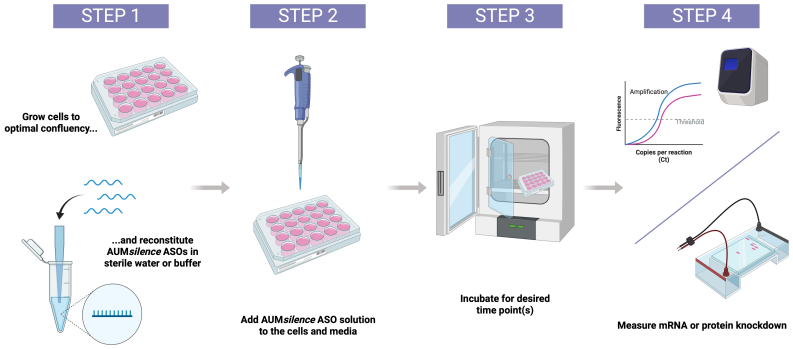
2. **Add AUM*silence* ASOs to the cells and media to the desired final concentration.**

   For silencing experiments, the working concentration of AUM*silence* ASOs can vary from **500 nM to 10 µM**. It is highly recommended to perform a dose response using 2-3 working concentrations **(500 nM, 5 µM and 10 µM)** to determine the most optimum concentration for your application. In some specific cases, **20 µM** or higher concentrations may be required.

   - For adherent cells: aspirate the growth media and overlay cells with media containing AUM*silence* ASOs or add AUM*silence* ASOs stock directly to the media overlaying the cells. Mix gently.

In Vitro Protocol

- For suspension cells, pellet the cells by low-speed centrifugation and gently resuspend the cell pellet in media containing AUM*silence* oligos. Alternatively, add AUM*silence* oligos directly to the media overlaying the cells.



3. **Incubate for the desired time point(s).**

4. **Analyze AUM*silence* ASOs treated cells after the desired time point (typically, 24 – 72 hours post treatment).**
   - **Note:** Uptake of fluorescently-labeled AUM*silence* ASOs can be observed as early as 4-8 hours, but full knockdown is best assessed at 24-96 hours post treatment.

Reference calculations: Amount of AUM*silence* ASOs

Cell culture plate	96-well	24-well	12-well	6-well
AUM*silence* ASO[1] stock (µL)	1 µL	5 µL	10 µL	30 µL
AUM*silence* 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 AUM*silence* ASO shown yields a final concentration of **1 µM using 100 µM 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 AUM*silence* ASO treatment. In general, a confluency of 30 – 50% is recommended at the time of AUM*silence* 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 AUM*silence* ASOs to the cell culture to maintain knockdown.
(3) AUM*silence* oligos can be fluorescently labeled (or with any desired label) to monitor cellular uptake.
Storage: (1) AUM*silence* ASOs are shipped in lyophilized form. Upon arrival, store them in -20°C. (2) When ready to use, resuspend AUM*silence* ASOs in sterile water or appropriate buffer at the desired concentration. Aliquot resuspended AUM*silence* ASOs in aliquots to avoid multiple freeze-thaw cycles.

Contact and customer support: For questions on products and protocols please reach out to us CustomerCare@aumbiotech.com or schedule a meeting with our scientific team here.