

Directions for Use

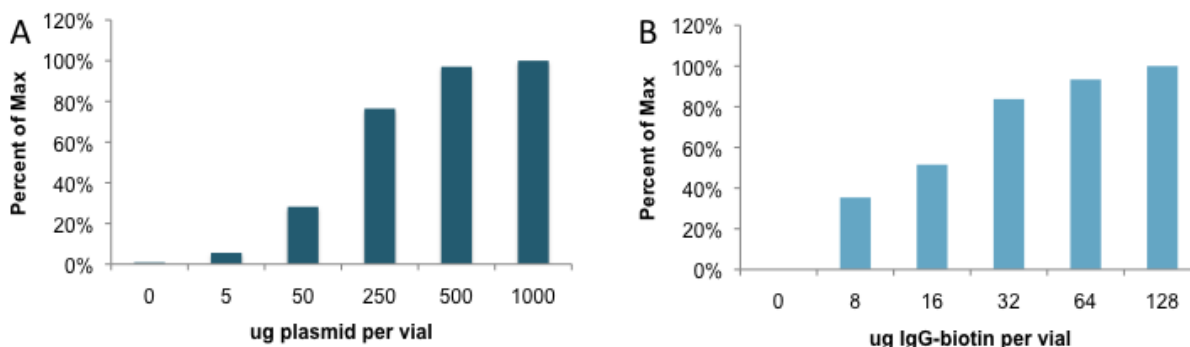
Targesphere[®] SA Targetable Acoustic Transfection Agent

Materials Needed

1. 1 vial Targesphere SA (TS-602)
2. 500 micrograms (µg) of plasmid (0.5-3.0 mg/mL) per vial of Targesphere SA
3. 100 µg of biotinylated IgG antibody targeting ligand per vial of Targesphere SA

Overview

Targesphere SA is a cationic delivery agent that can be used for molecularly targeted *in vitro* and *in vivo* transfection. The product is a dispersion of cationic microspheres, consisting of a core of biocompatible gas encapsulated by a lipid/polymer shell. The outer surface of the shell contains a layer of streptavidin. Plasmid, siRNA and other nucleic acid payloads can be coupled to the surface of the agent by simple incubation. The agent can be targeted for cell-specific transfection via a biotinylated targeting ligand bound to the streptavidin shell. Intracellular delivery of the payload is mediated by activation of Targesphere SA agents with acoustic energy¹, using an ultrasound scanner or sonoprotator.



Example 1. Loading curves for Targesphere SA with plasmid DNA and antibody targeting ligand. (A) GFP-coding plasmid (pMaxGFP, 3846 base pairs; Amaxa Biosciences) was added to Targesphere SA at increasing loading density and incubated for 25 minutes. Plasmid was then fluorescently labeled by incubation with YoYo-1 for 20 minutes, and samples were analyzed by flow cytometry. (B) Biotinylated rat IgG was incubated with Targesphere SA at increasing loading densities and incubated for 25 minutes. Un-bound antibody was removed by one round of centrifugal washing, and antibody density was quantified.

A. Targeting Ligand Conjugation Procedure

1. Conjugation of the targeting ligand should be performed prior to payload loading (Section B).
2. Disperse Targesphere SA by gently shaking the vial end-to-end for 10 seconds. The dispersion should appear uniformly opaque. Vortexing may be required to fully disperse the product.

3. Carefully remove the aluminum seal, and remove the stopper from the vial. Do not discard the stopper.
4. Add 50 µg of biotinylated antibody to the vial. When using non-antibody ligands, a conjugation ratio of 0.2 nmoles per 0.5 mL of Targesphere SA is recommended.
5. Replace the stopper and incubate the vial at room temperature for 20 minutes, with gentle agitation.
6. Targesphere SA is now ready for loading of the payload (Section B).

B. Payload Loading Procedure

1. Re-disperse Targesphere SA by gently shaking the vial end-to-end for 10 seconds.
2. Add the desired amount of payload to the Targesphere SA vial. Maximal loading density is approximately 500 µg for plasmid DNA per 0.5 mL vial. Optimal loading should be determined for each application.
3. Replace the stopper and incubate the vial at room temperature for 20 minutes, with gentle agitation.
4. Targesphere SA is now ready for *in vivo* or *in vitro* administration.

Dosage and Administration

Optimal *in vivo* dosage of Targesphere SA depends upon the tissue to be treated, route of administration, and sonoporation settings. Dose optimization should be performed for each application. In mice (~25 g), a dose of 30 microliters (µL) administered by jugular or retro-orbital injection generally results in detectible delivery to most tissues. Higher doses may be required for administration via tail vein.

In vitro dosage depends upon cell density and setup, and should be optimized for each application. In general, a dilution of 1:200 or less results in detectible payload delivery.

Sonoporation is best effected at low ultrasound frequencies (0.5 – 3 MHz) and high acoustic pressures. *In vitro*, a pressure of > 300 kPa is generally sufficient for sonoporation; higher pressures may be required for *in vivo* work. Please contact Targeson technical support for protocol assistance.

Stability and Precautions

Targesphere SA should appear as a milky white dispersion after re-dispersion by gentle shaking. Do not use if liquid in vial appears clear, as this indicates that the agent has been damaged or inactivated. Targesphere SA may be stored in intact vial at 4-8 degrees C. Targesphere SA does not contain preservatives or bacteriostatic agents. **Do not freeze.**

Once the Targesphere SA vial is opened and exposed to air, agents must be used within 8 hours. Store unopened vials at 4-8 °C.

References

1. Tlaxca JL, Anderson CR, Klibanov AL, Lowrey B, Hossack JA, Alexander JS, Lawrence MB, Rychak JJ. 2010. *Ultrasound Med. Biol.* 36(11): 1907-18.