

Protocol #1008

Preparation and Use of Targestar®-SA

v. 1.2

Materials Needed

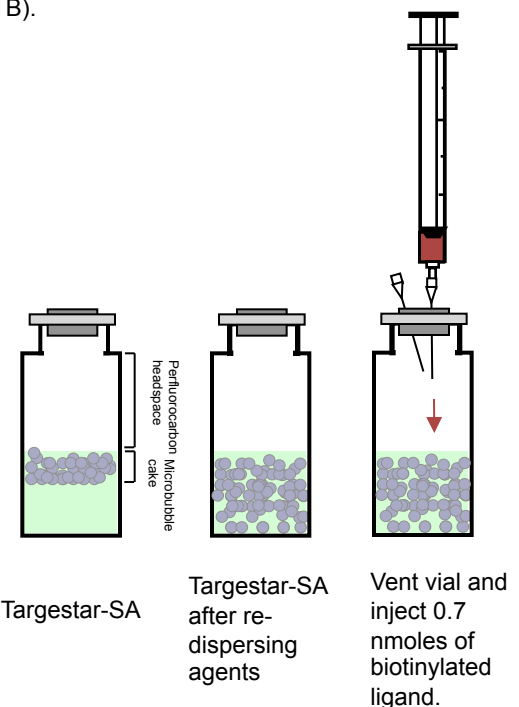
1. Targestar-SA ultrasound contrast agents (TS-201)
2. 0.7 nmoles of biotinylated ligand per vial of Targestar-SA
3. Targestar™ buffer or sterile saline for dilution

Overview

Targestar-SA is a microbubble ultrasound contrast agent that is coated with streptavidin. Biotinylated ligands, such as peptides, antibodies, and glycoconjugates, can be bound to the surface of the agents using biotin-streptavidin conjugation chemistry. After ligand conjugation, Targestar-SA is stable for up to 6 hours when stored at 4-8 degrees C. Excess biotinylated ligand that is not bound to the surface of the microbubbles may interfere with targeted microbubble adhesion *in vivo*; excess ligand can be removed from the preparation by centrifugal washing using the provided kit (step B).

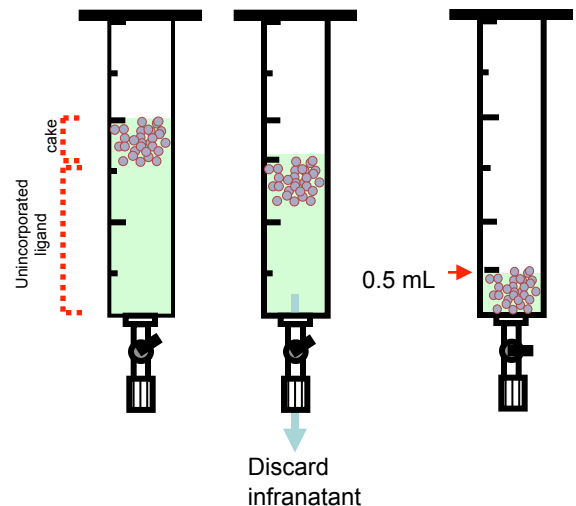
A. Ligand Conjugation

1. Disperse the Targestar-SA agents by gently shaking the vial end-to-end for 10 seconds. The dispersion should appear uniformly opaque.
2. Remove and discard the center of the aluminum crimp. Carefully insert a sterile 28-G needle through the elastomeric septum to vent the vial.
3. Using a syringe needle, slowly inject 0.7 nmoles of biotinylated ligand. For IgG antibodies, this is equivalent to ~100 micrograms. Increasing the amount of ligand may result in greater coupling yield.
4. Incubate the vial at room temperature for 20 minutes, with gentle shaking to keep the agents from caking.
5. If desired, unreacted biotinylated ligand may now be removed from the agents by centrifugal washing.



B. Removal of Unconjugated Ligand

1. Remove and discard the plunger from the provided 5 mL syringe. Attach the luer-lock stopcock to the syringe. Twist the stopcock to the closed position.
2. Open the Targestar-SA vial by carefully pulling the aluminium tear-off crimp. Re-disperse the agents by gently shaking the vial. Using a pipette, transfer the agents to the syringe/stopcock.
4. Withdraw 4 mL of Targestar buffer and add to the syringe/stopcock.
5. Centrifuge the syringe/stopcock at 400 X G for 4 minutes. The Targestar-SA agents will accumulate in a cake at the top of the syringe. If a cake is not observed or if the infranatant remains cloudy, repeat centrifugation.
6. Partially open the stopcock and slowly drain the infranatant dropwise, being careful not to disturb the cake. Discard the infranatant, and re-suspend the Targestar-SA agents in a total volume of 1.0 mL of Targestar Buffer or other aqueous buffer.



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7. Store the washed Targestar-SA in the original vial at 4-8 degrees C until ready for administration. The above procedure may be repeated, although the concentration of agent will be reduced approximately two-fold for each wash step.

Dosage and Administration

Optimal dosage of Targestar-SA depends upon the tissue to be imaged, route of administration, and scanner settings. Dose optimization should be performed for each application. In mice (25 g), a dose of 100 uL administered by jugular or retro-orbital injection generally results in sufficient contrast enhancement. Centrifugal washing of Targestar-SA will reduce the agent concentration, and may require higher doses. Higher doses may be required for administration via tail vein. Higher doses are generally required for ultra-high frequency (>20 MHz) imaging.

Contrast imaging settings (such as pulse inversion, or CPS) must be enabled on the ultrasound scanner for optimal contrast sensitivity. Accumulation of Targestar-SA at the target tissue, and washout of unbound agent, generally occurs over 5-15 minutes. After imaging, resident agent may be cleared by insonating the target tissue at high mechanical index (MI > 1.0). Please contact Targeson technical support for protocol assistance.

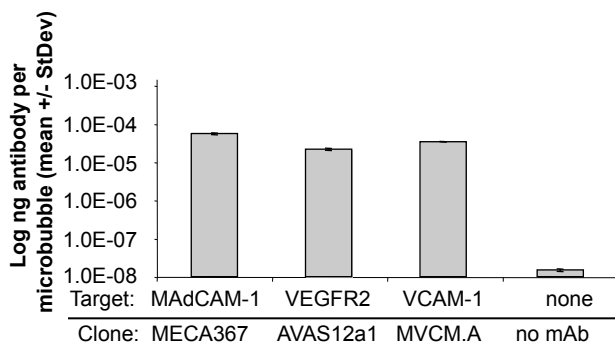
Precautions

Targestar-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 contrast agents have been damaged or destroyed. Targestar-SA does not contain preservatives or bacteriostatic agents. Do not freeze.

Application Examples

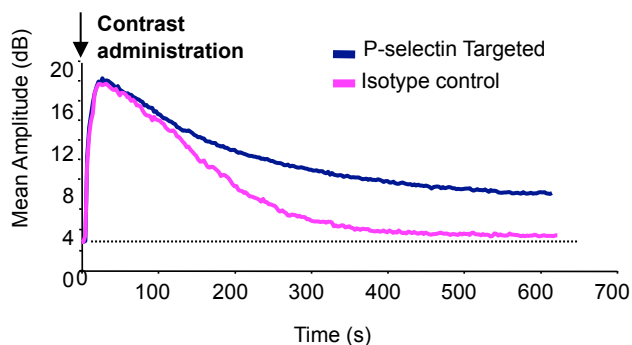
1. Conjugation of Biotinylated IgG Antibody to Targestar-SA

Targestar-SA was incubated with 100 ug of biotinylated antibody, and unreacted antibody removed by one round of centrifugal washing. Antibody surface density was measured by ELISA. This protocol resulted in an average conjugated antibody per microbubble of between 80 – 220 x10³ molecules.



2. Imaging P-selectin in a Mouse Model of Inflammation

A rat anti-mouse P-selectin antibody (clone Rb40.34; BD Biosciences) or IgG isotype control were used as targeting ligands. Inflammation was induced by subcutaneous injection of murine TNF-alpha two hours before imaging. Microbubbles were administered retro-orbitally, and accumulation within the hindlimb was visualized over 10 minutes at 8 MHz, MI=0.25 (CPS on Siemens Sequoia). Accumulated agent was cleared between doses by insonating the hindlimb at MI=1.2 for 1 minute. Dotted line shows precontrast baseline.



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