

# Protocol #1002

## Preparation and Use of Targestar®-B

v. 3.1

### Materials Needed

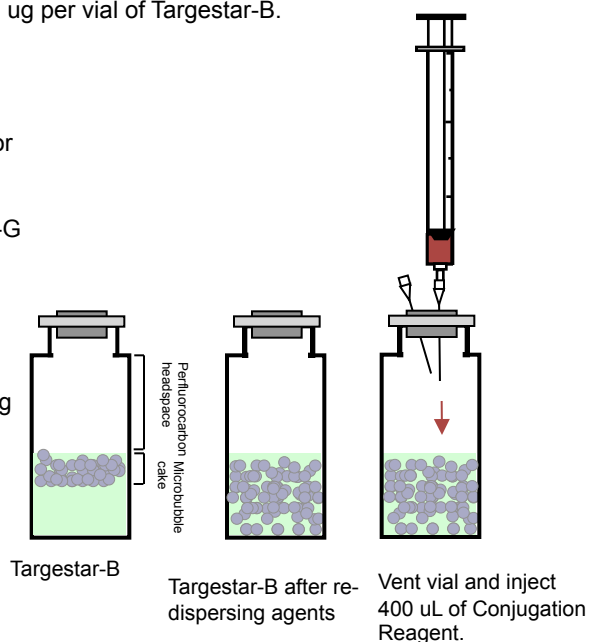
1. Targestar-B ultrasound contrast agent Kit (TS-107)
  - Targestar-B biotin contrast agents (1.5 mL vial)
  - Targestar Conjugation Reagent (400 uL)
  - Targestar Buffer
  - Syringe/stopcock washing apparatus
2. Three (3) nmoles of biotinylated ligand per vial of Targestar-B

### Overview

Targestar-B is a microbubble ultrasound contrast agent that is coated with biotin. The agent can be subsequently derivatized with streptavidin, using the provided conjugation kit. Biotinylated ligands, such as peptides, antibodies, and glycoconjugates, can then be bound to the surface of the agents by biotin-streptavidin conjugation chemistry. After ligand conjugation, Targestar-B is stable for up to 6 hours when stored at 4-8 degrees C. If desired, fluorescent or otherwise labeled streptavidin can be substituted for the Targestar Conjugation Reagent (Step A.3) at a concentration of 450 ug per vial of Targestar-B.

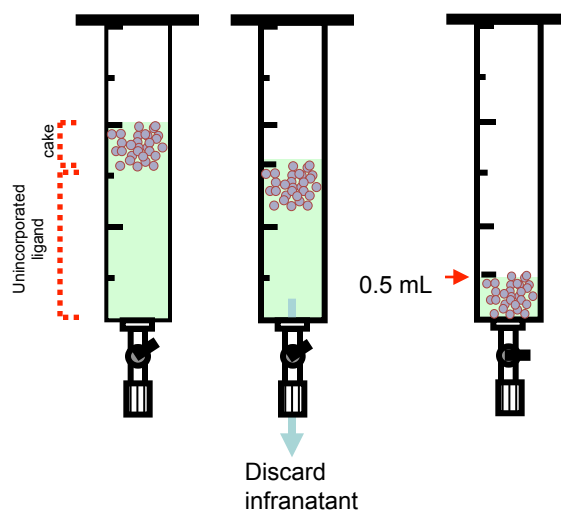
### A. Agent Preparation

1. Disperse the Targestar-B agents by gently shaking the vial end-to-end for 10 seconds. The dispersion should appear uniformly opaque.
2. Fold back 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 400 uL of Targestar Conjugation Reagent.
4. Incubate the vial at room temperature for 20 minutes, with gentle shaking to keep the agents from caking.



### B. Removal of Unconjugated Coupling Agent

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-B vial by carefully pulling the aluminum tear-off crimp. Re-disperse the agents by gently shaking the vial. Using a pipette, transfer the agents to the syringe/stopcock.
3. Withdraw 4 mL of Targestar buffer and add to the syringe/stopcock.
4. Centrifuge the syringe/stopcock at 400 X G for 4 minutes. The Targestar-B 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.
5. 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-B agents in a total volume of 1.0 mL of Targestar Buffer or other aqueous buffer. Transfer the washed Targestar-B to the original vial.



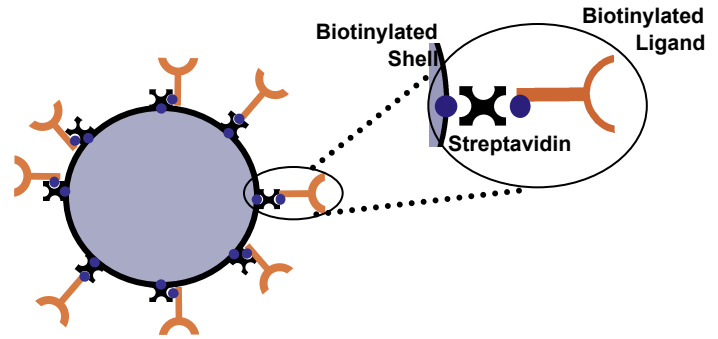
## C. Ligand Conjugation

1. Add 3.0 nmoles of biotinylated ligand. For IgG antibodies, this is equivalent to ~450 micrograms. Increasing the amount of ligand may result in greater coupling yield.

2. Replace the stopper on the vial, and incubate at room temperature for 20 minutes with gentle shaking to keep the agents from caking.

3. After incubation, remove unreacted biotinylated ligand by centrifugal washing, as in section B.

4. Re-suspend Targestar-B in 1.0 mL of Targestar Buffer. The final Targestar-B particle concentration is  $2 - 5 \times 10^8$  microbubbles per mL. Additional washing steps may be performed to further reduce the concentration of unconjugated ligand.



## Dosage and Administration

Optimal dosage of Targestar-B 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  $\mu$ L administered by jugular or retro-orbital injection generally results in sufficient contrast enhancement. Multiple centrifugal washing rounds will reduce the Targestar-B 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-B 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.

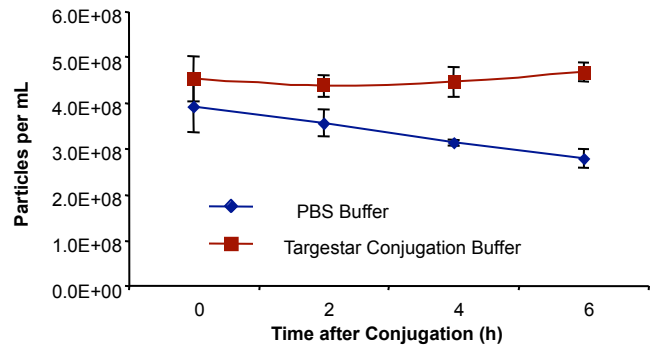
## Precautions

Targestar-B 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-B does not contain preservatives or bacteriostatic agents. Targestar-B is stable for 6 months when stored at 4-8 degrees. Once conjugated, Targestar-B should be used within 6 hours. Do not freeze.

## Application Examples

### 1. Stability of Conjugated Targestar-B

Targestar-B was prepared and stored on ice. As a control, PBS was used as a wash buffer. Microbubble concentration was measured over six hours using electrozone sensing.



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