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Protocol

Preparation Protocol for the **Target-Ready Vevo MicroMarker[®]** Contrast Agent



System Compatibility: This guide contains instructions and suggestions for work on the Vevo2100, VevoLAZR, Vevo 3100 systems and transducers from the MS, MZ and MX series.

Introduction

The Vevo MicroMarker contrast agents are made by Bracco Research SpA and used to enhance tissue and vascular imaging exclusively on the VisualSonics' Vevo high-frequency ultrasound systems.

- Vevo MicroMarker agents are lyophilized microbubbles with a lipid-based outer shell that contain polyethylene glycol, phospholipids and fatty acids
- They are stored in a glass vial containing a gas head-space consisting of nitrogen and perfluorobutane (C₄F₁₀)
- The microbubbles will become gas-filled agents when reconstituted with saline
- Vevo MicroMarker contrast agent kits can be stored at 4°C (39.2°F) and have a shelf life of 12 months
- Vevo MicroMarker contrast agents that have been opened and reconstituted are stable within the vials for 3 hours
- These untargeted microbubbles have a clearance time of approximately 10-20 minutes in mice provided that optimized dosing is used.

Objective

The objective of this protocol is to outline instructions for the preparation of the Target-Ready Vevo MicroMarker contrast agent to be administered via bolus injections or via an infusion pump.

*****Note***:** *This is a generic protocol describing the preparation of the contrast agent and bolus injection to be injected into the animal. Refer to application-specific protocols for details about imaging using the Vevo systems.*

This protocol is intended for mouse imaging applications only.

Materials needed for the preparation of Targeted-Ready Contrast Agent

- 8x1 mL syringe
- 6x21G 5/8" needle
- 3x27G 1/2" needle
- Sterile saline vial
- Vevo MicroMarker Target-Ready Contrast Agent – 2 vials

Additional materials not provided and may be required include:

- IV Cannulation device
- Biotinylated antibody of choice and isotype control
- Eppendorf tubes
- Pipettes and tips
- Infusion Pump – the following vendors have various models of suitable pumps available:
 - www.syringepump.com
 - www.harvardapparatus.com

Note: VisualSonics has tested the Harvard Apparatus Pump 11 Elite Nanomite with good results.

Preparation

A *Prepare Vevo MicroMarker Contrast Agent Conjugated with the Isotype Control Antibody*

1. Attach the 21G 5/8" needle to the 1 mL syringe;
2. Reconstitute the Target-Ready contrast agent from one of the vials by injecting 0.7 mL of saline. Remove the syringe and leave the needle in the vial to vent for a few seconds, and then remove the needle completely.\
3. Gently agitate for 10 seconds and let sit at room temperature for 5 minutes.
4. Dilute 20 µg (depending on experiment) of the biotinylated isotype control antibody to a total volume of 300 µL with saline. For example, add 40 µL from a 0.5 mg/ml antibody stock to 260 µL of saline.
5. Using the appropriate pipette, add 260 µL of saline to an eppendorf tube, and then pipette 40 µL (20 µg) of antibody into the same tube.
6. Using a 21G needle with a 1 mL syringe draw up the entire content of the tube and inject into the vial of reconstituted Vevo MicroMarker (the total volume of the prepared contrast agent will be 1000 µL).
7. Gently agitate the vial by hand for 1 min and let it rest at room temperature for 15 minutes.

B *Prepare Vevo MicroMarker Contrast Agent Conjugated with Primary Antibody*

1. Attach the 21G 5/8" needle to the 1 mL syringe;
2. Reconstitute the Target-Ready contrast agent from one of the vials by injecting 0.7 mL of saline. Remove the syringe and leave the needle in the vial to vent for a few seconds, and then remove the needle completely.\
3. Gently agitate for 10 seconds and let sit at room temperature for 5 minutes.
4. Dilute 20 µg (depending on experiment) of the primary antibody to a total volume of 300 µL with saline and inject it into the vial of reconstituted Vevo MicroMarker as described at step 2 above.
5. Gently agitate the vial by hand for 1 min and let rest at room temperature for 15 minutes.

C Preparation of the Flush Syringe

1. Attach a 27G 1/2" needle to a 1 mL syringe filled with sterile saline.

Guidelines for Injection of Vevo Targeted-Ready Contrast Agent

A Injection of Vevo MicroMarker Contrast Agent Conjugated with Isotype Control Antibody

1. Gently agitate the vial in a top-to-bottom manner before collecting a sample in order to ensure even distribution of the microbubbles in suspension. Using a 1 mL syringe and a 21G 5/8" needle draw up approximately 120 μ L of prepared Isotype Control conjugated Vevo Target-Ready contrast agent from the vial (70 μ L of this volume will compensate for the dead space in the needle hub). After removing the needle and syringe from the vial, draw all of the liquid into the syringe. Ensure that all 120 μ L of contrast agent is withdrawn into the syringe, leaving nothing in the needle hub.
2. Replace the 21G 5/8" needle with a 27G 1/2" needle to prepare for bolus injection. Remove all air from the syringe and adjust the volume to be delivered to 50 μ L. This is the bolus volume for the isotype control conjugated agent to be injected into the cannulation set-up (the "isotype control syringe").

B Injection of Vevo MicroMarker Contrast Agent Conjugated with Primary Antibody

1. Gently agitate the vial in a top-to-bottom manner before collecting a sample in order to ensure even distribution of the microbubbles in suspension. Using a 1 mL syringe and a 21G 5/8" needle draw up approximately 120 μ L of prepared primary antibody conjugated Vevo Target-Ready contrast agent from the vial; the extra 70 μ L volume will compensate for the dead space in the needle hub. After removing the needle and syringe from the vial, draw all of the liquid into the syringe. Ensure that all 120 μ L of contrast agent is withdrawn into the syringe, leaving nothing in the needle hub.
2. Replace the 21G 5/8" needle with a new 27G 1/2" needle to prepare for bolus injection. Remove all air from the syringe and adjust the volume to be delivered to 50 μ L. This is the bolus volume for the primary antibody conjugated agent to be injected into the cannulation set-up (the "target of interest syringe"). Higher injection volumes will lead to increased signal and may be required for some applications.

*****Note***:** The prepared contrast agent is most stable in the perfluorobutane gas headspace within the vial, so bolus volumes should only be removed just prior to delivering the injection.

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