USER MANUAL

Microbubble Reagent for Contrast Ultrasound Applications

VesselVue™ P-formulation

Part number:	P-4007003
Vial Size:	3 mL
Volume:	1 mL

Storage: 4°C

Version: 2.1

For research only. Not for use in diagnostic procedures

VesselVue

Microbubble Regent for Contrast Ultrasound Applications

Caution: For Research Use. This product is intended for animal research only and not for use in humans.

Part Number: P-4007003
Use with Revvity's Bead Ruptor™ VesselVue™ Homogenizer, Part Number: 25-030

Product Description

VesselVue™ formulations are microbubble contrast reagents for contrast enhanced ultrasound (CEUS) imaging and preclinical therapy applications. VesselVue microbubbles can enable the visualization and quantification of tumor or organ vascularity in CEUS imaging, revealing dynamic changes in blood vessels for disease characterization and therapeutic response studies. In addition to diagnostic research applications, some labs have also used VesselVue microbubbles as either a catalyst or a delivery modality to interrogate several different ultrasound-enabled therapeutic applications in preclinical studies.

P-formulation (Polydisperse):

- Has a wider microbubble size distribution, and a smaller mean diameter.
- This formulation is an excellent all-around vascular imaging contrast agent for general CEUS imaging applications.
- This formulation can provide improved imaging signal sensitivity for specific CEUS applications. The larger bubble size also increases the circulation time for bolus injections, extending the imaging window.

This guide is for preparing the VesselVue P-formulation agent for use in traditional CEUS imaging, or in conjunction with Acoustic Angiography, a CEUS imaging mode exclusive to the Vega® Automated Ultrasound Imaging System, to help users achieve high-sensitivity *in vivo* ultrasound imaging of microvasculature.

Each 3 mL vial contains 1 mL of P-formulation contrast agent, sufficient for imaging approx. 5-20 mice (weight ~20 grams each) when diluting 1:1 with saline via either a bolus or continuous infusion I.V. injection.

Storage and Handling

VesselVue vials are shipped on cold gel packs. Immediately after arrival, store vials at 4° C until ready to use. Never freeze.

When stored as directed, VesselVue P-formulation remains viable for a minimum of 6 months from the date of shipping.

VesselVue is best when used within 24 hours after septum has been pierced. Vials can be used for multiple injections within a single imaging study. Pierced vials should not be stored. Discard unused portion.

Administration Methods and Preparation

Decide in advance which administration method you will use.

Here we briefly describe two methods. Most studies in literature use tail vein infusion for microbubble contrast agents in rodents. Some studies have also reported using I.V. injection via other peripheral veins.

I. Bolus

The half-life, or circulation, time for P-formulation microbubbles is 2-5 minutes when using bolus injections [Sirsi 2010]. The VesselVue contrast solution is diluted1:1 with saline for a final volume of 100 µL per mouse. Maximum volume is determined by your lab's specific IACUC protocol.

- A larger dose can be injected initially to extend the imaging window. However, too many microbubbles in circulation may result in decreased image quality, as the layer of microbubbles will "shadow" regions of tissue below the skin's surface.

II. Continuous Infusion

For continuous infusion, a "tail vein catheter" consisting of a syringe connected to a thin gauge of medical grade plastic tubing is coupled to the tail vein of a mouse or rat. The syringe contains VesselVue microbubbles diluted 1:1 with saline.

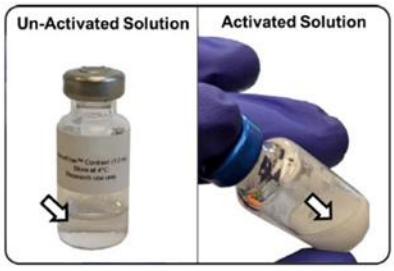
- For guidance on creating tail vein catheters, please contact our Global Technical Support Team: LS.ReagentsTechSupport@revvity.com

More detail is provided on performing each of the two methods in the following pages of this protocol.

1. Preparing Mice for Imaging

The fur on and around the areas of the animal that are to be imaged should be depilated prior to microbubble injection. Nude mice, or immunocompetent hairless SKH1-E mice, do not require depilation; however conventional strains of haired mice, like BALB/c or C57BL/6, require depilation.

2. Preparing P-formulation Microbubbles



Above: P-formulation agent shown before and after activation

VesselVue P-formulation microbubbles are shipped un-activated, meaning you must activate them immediately prior to use with the Bead Ruptor VesselVue Homogenizer.

Activation should only be performed once on an unopened vial just prior to use. Allow the vial to warm to room temperature before placing in homogenizer.

Below: The Bead Ruptor VesselVue Homogenizer, 25-030

To activate the microbubbles, insert an unopened vial (unpierced septum) into the Bead Ruptor VesselVue Homogenizer vial holder and close the cover. Vial needs to shake for 90 seconds. Adjust timer to 90 seconds if needed. Press the Start button.

When mixing cycle is complete, remove the vial from the homogenizer and let the vial sit for 5 minutes to allow the microbubble concentration to stabilize. Then proceed to administration protocol below. For more information, refer to the Bead Ruptor VesselVue Homogenizer Quick Start Guide.

3. Administration and Imaging Protocols

P-FORMULATION AGENT *MUST BE ACTIVATED* BEFORE PROCEEDING TO EITHER ADMINISTRATION METHOD BELOW.

A 1:1 microbubble/saline ratio is typically used for the Acoustic Angiography imaging mode on the Vega ultrasound system. Other dilutions have been published and can be tailored to your specific instrumentation or research application.
Note that when withdrawing stock microbubbles during the dilution process, care must be taken to avoid damaging the microbubbles by introducing sudden changes in pressure (vacuum or compression).

Microbubbles should be diluted with sterile saline immediately prior to use.

I. Preparation for a Bolus Administration

A bolus of microbubbles can be injected through the tail vein.
Using a 1:1 microbubble/saline solution, the recommended total volume is:

- 100 µL per mouse (20 g)
- 150 µL per rat (~150 g)

1. Allow the vial to warm to room temperature before using the VesselVue mixer, and let the vial sit for 5 minutes after mixing to allow the microbubble concentration to stabilize.
2. Add appropriate amount* of sterile saline to a 1- 5 mL syringe using a 20g needle or similar (*Maximum volume is determined by your lab's specific IACUC protocol). Wait until shortly before imaging to continue to next steps.
3. While the VesselVue vial is upright, insert a venting needle into the septum. The vent needle is critical to equalize the internal air pressure with the atmosphere. (Fig 1A)

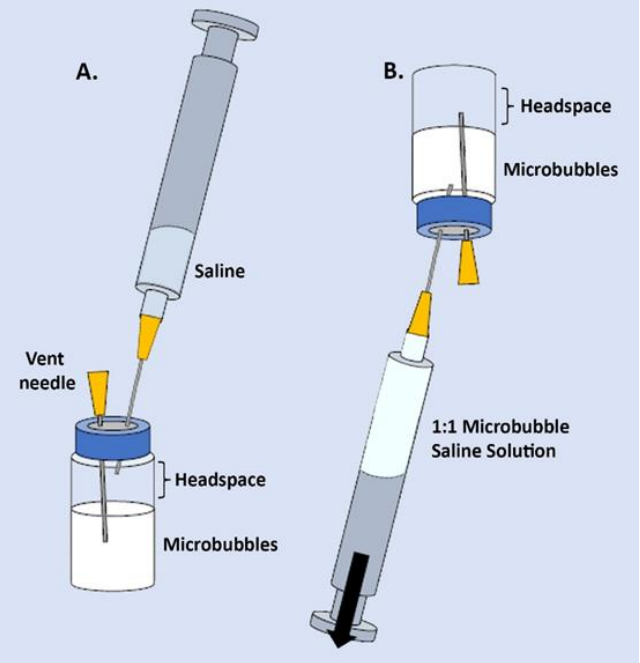


Figure 1. (A) Insert two needles into the rubber septa. (B) Invert the vial and draw the contrast agent into the syringe while allowing the vial to vent to prevent vacuum formation. Failure to prevent vacuum in the vial will destroy microbubbles.

4. Insert the extraction needle connected to the saline syringe into the septum next to the venting needle. (Fig. 1A)
5. Invert the vial and ensure that the tip of the venting needle reaches above the microbubble/gas interface. (Fig 1B)
6. Draw the appropriate volume of microbubbles into the syringe, ensuring that the tip of the injection needle remains below the microbubble/gas interface. (Fig 1B)
7. Mix solution by gently rolling the syringe between the palms of your hands. DO NOT mix by pumping the syringe.

II. Preparation for a Continuous Infusion Administration

Suggested Materials:

- 1:1 diluted VesselVue P-formulation microbubble contrast agent (Minimum Calculated Volume)
- VesselVue Microbubble Mixer
- Catheter set, including thin gauge medical grade plastic tubing
- Sterile saline for priming the catheter to prevent air from being introduced into the vein, and to confirm successful needle placement in vein
- 2 syringes: one for normal saline and one for the diluted VesselVue contrast reagent
- Medical tape or other biocompatible adhesive to secure the needle placement at the injection site
- Syringe pump for administration of diluted VesselVue

1. Allow the vial to warm to room temperature before using the VesselVue mixer, and let the vial sit for 5 minutes after mixing to allow the microbubble concentration to stabilize.
2. To set up catheter infusion line, prepare one syringe containing saline and one syringe containing 1:1 microbubble dilution mounted on a syringe pump with correct settings for speed. Place pump close to instrument to keep length of the catheter short. Keep the overall length of the catheter tube as short as possible to reduce the volume of saline in the catheter.
3. Prime the tubing with sterile saline by manually injecting enough saline to fill the catheter. Also, take this opportunity to estimate the dead space in the catheter set (i.e. the volume in the needle and tubing combined).
4. Connect the syringe containing the calculated volume to the pre-filled tubing coupled to the animal's tail vein via a needle. Secure the tubing in place and start the pump.
5. Wait approximately 30 seconds before starting image acquisition in order for the microbubbles to move through the tubing and achieve a steady state level of microbubbles in the animal's vasculature (perfusion time).

Minimum Calculated Volume

The calculated volume of microbubbles and saline needed for infusion per mouse is determined by three variables. These are:

1. Dead-space in syringe and catheter (catheter volume)
2. Estimated scan time
3. Imaging subject (mouse or rat)

1. Dead-space in syringe and catheter (catheter volume)

    a. The catheter volume can be determined using saline and will depend on the length of tubing used.

    Note: be mindful of the length of the tubing, as a non-negligible amount of microbubble solution will occupy the tubing and decrease the microbubble solution volume available for imaging in longer imaging studies. Longer tubing also increases the pressure in the tube which could damage the bubbles.

2. The estimated scan time is determined by the size of the area to be scanned (larger area = longer scan time) and the preset mode selected in the software. The modes have a corresponding length of time needed to scan for that selected preset.

    a. The Vega Ultrasound Imaging System's software, SonoEQ, includes 2 parameter "presets" for Acoustic Angiography depending on your imaging goals:

        Density: captures signal from both sub-resolution and resolvable vessels.
        Morphology: eliminates sub-resolution signal to highlight resolvable vessels.

    An estimate of the scan time required for each preset is provided in SonoEQ (Please see the SonoEQ user manual).

3. Whether the imaging subject is a mouse or a rat. Infusion rates are:

    - 15 µL/min per mouse (20 g)
    - 40 µL/min per rat (~150 g)

Example of Minimum Calculated Volume:

Using the infusion rate for a mouse, the following example applies to a Morphology preset scan that takes 4 min and requires 100 µL to fill the catheter.

(15 µL/min mouse injection rate) * (4.0 min scan time)
+ (15 µL/min mouse injection rate) * (0.5 min perfusion time)
+ (100 µL pre-fill catheter volume)
= ~170 µL total volume injected per mouse

Note: 170 µL total volume is 85 µL saline and 85 µL of microbubbles, assuming a dilution of 1:1.

For further information on *in vivo* imaging and related products, please contact your local Revvity representative or visit: https://www.revvity.com

For Technical Support, please contact: **LS.ReagentsTechSupport@revvity.com**

www.revvity.com

revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com