PRODUCT DATA SHEET

Vascupaint™ Silicone Rubber Injection Compounds

Description

Vascupaint™ lead-free silicone injection compounds fill and opacify the microvasculature structures for 3D ex vivo imaging using micro-CT, dissection or brightfield microscopes, or SWIR fluorescence imaging.

The injection compounds are available in two different colors including yellow (SKU# MDL-121) for micro-CT imaging and green (SKU# MDL-122) for NIR or SWIR fluorescence imaging as well as clear (SKU# MDL-123) for your custom preparations. Other colors are available upon request.

Vascupaint™ is a less viscous alternative to conventionally used lead-based injection compounds making it easier to perfuse non-surviving organisms as well as human organs for ex vivo 3D vascular cast analysis.

Features & Benefits

- X-ray attenuation of bismuth nanoparticles in Vascupaint yellow product allows for high resolution micro-CT imaging
- The sub 750 nm particle size of the bismuth particles in yellow product allows for adequate filling of capillaries and microvasculature
- Low viscosity of mixture allows for perfusion into non-surviving organism at physiological pressures and complete filling of microvasculature
- The catalyst is a defined media that uses water molecules to drive cross-linking of monomer. The bottle of catalyst does not require shaking prior to use and there is no settling of cross-linker and catalyst and the catalyst bottle does not require shaking prior to use.

Applications

- Vascular corrosion casting, imaging of angiogenesis, quantification of vascular volumes in ischemia and tumors, microCT analysis of vascular morphometry, assessing vascular architecture of organs, teaching adjunct, visualization of microcirculation for surgery techniques

Vascupaint Kit Components

- 1 bottle of silicone (Green, Yellow, Lime, Blue, Red or Clear), 200 ml
- 1 bottle of diluent, 200 ml
- 1 bottle of catalyst, 20 ml

Storage

All components of this kit should be stored at room temperature and are stable for one year from date of manufacture

Product Safety and Handling

This product is for R&D use only, not for drug, household, or other uses. Please review the material safety datasheet (MSDS) for proper safety and handling procedures.

Preparing Stock Solutions of Silicone and Diluent Mixtures

It is possible to prepare stock solution of the silicone and diluent compound mixture based on the ratios used in your experiments, for example the ratios mentioned in point 1 of the protocol example below. For example, you can add 80 ml of the silicone to 100 ml of the diluent and store for future use.

Using Previously Prepared Stock Solutions of Silicone and Diluent Mixtures

Some 'soft settling' of pigments will occur in the stock solution. You can remove this by gently inverting the bottle several times. The pigments will remain suspended in solutions for several hours after the gentle inversion of the stock solution bottle.

Protocol Example

A recommended starting protocol for mouse perfusions is as follows:

1. Gently invert and/or shake the silicone bottle and then transfer some of it's contents to a 50 ml

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conical tube. In addition, transfer some of the contents of the diluent bottle to a 50 ml conical tube and place both tubes in rack holder. Gently invert the conical tube with silicone a few times and then mix all kit components in a conical tube with the following ratios:

- 4 ml silicone
- 5 ml diluent
- 0.45 ml catalyst (5% Catalyst to mixture)

Other ratios have been used depending on the perfusion procedure (retrograde or anterograde), the organ of analysis and desired stiffness of silicone in the vascular cast.

Transfer the mix to a petri dish for aspiration into a 10 ml syringe with a blunt 20 or 27-gauge needle. Make sure there are no air bubbles when aspirating the mix.

2. To anesthetize mouse, you can inject 100 mg/kg ketamine with 10 mg/kg xylazine diluted in saline via IP route for anesthesia..Make sure it is completely unresponsive. Then place mouse on surgical station (tray, cork board and blue absorbent pad), tape the mouse on the pad to prevent it from moving during injection and spray the body of mouse with ethanol.

   Alternatively, inject the heparin (app. 0.05 ml) IP a few minutes before or simultaneously with the anaesthetic. It is best to use more anaesthetic than less so as to prevent coagulation. Once heparinized, the mouse can wait for some minutes (you do not have the stress to do it as quick as possible).

3. Cut abdominal cavity open; avoid cutting or disturbing any internal organs.

4. For brain imaging procedure, gently separate the liver from the diaphragm.

5. Cut open the diaphragm to gain access to the chest cavity and unrestricted access to the descending aorta.

6. Two proposed methods of cannulation :

   (1) Cannulate the major supplying artery (in case of mouse – it is usually aorta). Cannulate in antegrade direction for the hindlimbs or kidneys and in retrograde direction for for brain/eye. Two ligations can be used for in situ fixations.

   (2) cut the needle tip to make it blunt before insertion into the heart Insert the syringe needle into heart through the left ventricle and all the way up to aorta. Cut open the right atrium to allow blood and contrast agent to flow out of circulation. It is also possible to cut the edges of the liver instead of the atrium to ensure that the outflow of the contrast occurs less rapidly. Some users have found that clamping away the non-needed branches (e.g. abdominal aorta below renal arteries in case of kidney perfusion or mesenteric vessels for perfusion of the hind limbs or the left ventricle for the brain increases the quality of the perfusion

   See example video of this procedure. Ref. https://youtu.be/Icm1OG-vUl4

7. If IP injection of heparin was not performed in step 2, Perfuse with heparinized PBS (5ml) first, and then switch to a syringe, which has been filled with formalin (5 ml) for fixation. Following fixation, switch to the syringe with the vascupaint mixture. Before injecting Vascupaint, put a hemostat to clip on the heart to hold the needle in place and add crazy glue to make sure the clip is held in place and that a back-pressure is sensed when introducing the mixture into the organism. By doing so, the needle will be fixed in the aorta and leakage of Vascupaint mixture out of the heart will be minimized, if not completely avoided. A perfusion pump can be used to administer Vascupaint mixture slowly to avoid any leakages or disruptions of the hemostat clamp.

8. Note: avoid creating air bubbles at the tip of the needle while switching syringes.

9. Leave the mice in fridge overnight. For brain perfusion procedures, leave the mice upside down at 4ºC, overnight.

10. The following day, the Vascupaint inside the mouse's body should be hardened and organs can be removed carefully. For brain procedures, the head and exposing the skull. The organ can be further processed using standard tissue digestion techniques for analysis with dissecting

or brightfield microscopy (*this application may require increased level of catalyst to ensure proper hardening of the Vascupaint). The intact organ is ready for analysis with microCT imaging for the Yellow version of Vascupaint and surface vessels can be assessed with visual inspection or microscopy to determine success of perfusion.

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