eXIA™ 160*XL*

PLEASE READ ENTIRE DOCUMENT BEFORE STARTING

- **Do NOT Freeze**
- **Store between 4° to 18° C**
- **Protect from light**
- **For Research Use ONLY**
- **Not for Use in Humans**

INTRODUCTION

Microcomputed tomography (micro-CT) for morphological and functional analysis of animal models of human disease has evolved dramatically in the last decade as a powerful tool for monitoring disease progression and the efficacy of novel therapeutics. Moreover, dual-modality PET/CT, SPECT/CT, and Optical/CT systems have been widely adopted for preclinical research using a variety of imaging approaches. However, due to the insignificant difference in X-ray attenuation within soft tissues and blood vessels, non-contrast imaging is of a limited value. Intravascular injection of small water-soluble iodinated radiographic contrast media (RCM) is the most commonly used clinical method to opacify blood vessels. Following injection, contrast agents are distributed rapidly between circulating blood and other extracellular fluids, which to a certain extend also allows visualization of anatomic structures of the perfused organs. Water-soluble iodinated contrast agents are excreted mainly through the kidneys following intravascular administration, and in the absence of renal dysfunction, have very short elimination half-lives. While such transient changes in the levels of contrast within body compartments are not critical in clinical settings due to the short imaging times of clinical CT systems, these changes can significantly impair data quality acquired with slow micro-CT scanners. Thus, for micro-CT studies it is necessary to use long-residence-time vascular and organ-specific contrast agents to improve soft tissue imaging.

To improve non-invasive soft tissue micro-CT imaging, a highly opacifying iodine-based eXIA™160*XL* contrast with eXtra-Lasting (*XL*) blood-pool phase was developed. In contrast to conventional iodinated contrast agents that rapidly diffuse out from the vascular compartment, eXIA™160*XL* stays in the blood circulation for a prolonged period of time until it becomes metabolically hydrolysed and excreted. The prolonged vascular circulation of eXIA™160*XL* allows several important applications in heart and vascular imaging when a relatively long scanning time (e.g. that involves cardiac or respiratory gating) or follow-up studies with close time intervals are required. After several minutes in the circulation, eXIA™160*XL* is taken up by metabolically active organs and metabolized. Thus, in addition to blood pool imaging, eXIA™160*XL* can opacify metabolically active organs such as the liver, spleen, brown adipose tissue (BAT), myocardium, enabling successful imaging of these organs for monitoring disease progression and the efficacy of therapeutic interventions.

APPLICATIONS

eXIA™160*XL* is an aqueous colloidal polydisperse contrast material with eXtra-Lasting (XL) blood-pool phase suitable for parenteral administration. It contains 160 milligrams (mg) of iodine per millilitre (mL) as an X-ray attenuating agent. eXIA™160*XL* was designed to address a broad spectrum of micro-CT applications alone or in conjunction with PET, SPECT, bioluminescence, and fluorescence *in vivo* imaging. After intravenous administration, iodinated colloidal particles of eXIA™160*XL* avoid renal filtration and diffusion from the intravascular into the interstitial space, thereby providing long-lasting vascular enhancement and thus the opportunity of using it as a blood pool contrast agent. Following intra-vascular injection, eXIA™160*XL* is rapidly diluted in the vascular compartment (plasma) and the degree of contrast enhancement is directly proportional to the amount of iodine in the administered dose. While a large volume injection of eXIA™160*XL* will provide superior levels of intravascular contrast, to satisfy both scientific and regulatory requirements, excessively large volumes should be avoided. Excess volumes of solutions administered intravenously can trigger hemodynamic changes and occurrence of pulmonary oedema, cardiovascular failure and be lethal. The consensus figures based on published literature and internal guidelines limit the maximum intravenous rapid bolus volume to 5 mL/kg of body weight in mice and rats. Larger volumes can be administered provided the rate of injection is kept slow and precautions are taken to avoid getting the solution outside the vein. Considering that the circulating blood volume in a mouse and rat is on average 72 and 64 mL/kg of body weight respectively, i.v. administration of 0.1 mL of eXIA™160*XL* to a 20-g mouse (equivalent to 800 mg iodine/kg body weight), will produce ~10 mgI/mL in the vascular compartment immediately after injection. Although this dose can be reliably distinguished from avascular structures on most micro-CT systems, it is recommended that the optimal dose for a particular animal model, micro-CT instrument, instrument settings, and image processing is determined experimentally.

Due to the product's propensity to opacify metabolically active organs at later time points after the contrast administration, eXIA™160 *XL* can prove useful for the non-invasive *in vivo* detection of liver and spleen abnormalities, myocardial infarction and cardiomyopathy, and to identify and quantify active brown adipose tissue (BAT), similarly to 2-Deoxy-2-[F]fluoro-D-glucose (FDG) positron emission tomography (PET), but without the need for a radioactive tracer.

The time-dependent organ enhancement values obtained following a single bolus administration of eXIA™160*XL* indicate that the peak contrast level in blood vessels occurs immediately after injection. For more details on time-dependent organ enhancement, please read the product related publications shown below.

ADMINISTRATION

Mice are the most widely used animals for a wide range of pre-clinical imaging experiments. eXIA™160*XL* can be administered via a lateral tail vein of a laboratory mouse as a single bolus injection as provided. Low dead space ½ CC U-100 28G½ Insulin Syringes (Becton Dickinson and Company, #329461) are recommended for eXIA™160*XL* administration in mice. Good animal handling and restraint is the most important technique for correct administration and self-protection, as minor injuries through mouse bites, mainly into fingers, may occur even in experienced personnel. The wearing of a double layer of synthetic gloves has a considerable potential to reduce the number of bites that perforate gloves. In case of the occurrence, the bite wound has to be immediately cleansed and disinfected in order to prevent infection. Physical restraint should be performed on conscious animals, however, to minimize stress, slight sedation or anaesthesia can be used to administer the product, especially when volumes greater than 5 mL/kg of body weight need to be slowly infused. Before entering the vial with a needle, resuspend the content of the vial by gentle inversion. Expose and treat the rubber of the vial stopper using 70% alcohol. Enter the vial with the needle and withdraw a required volume of the agent. The volume of eXIA™160*XL* should be based on the animal body weight and sometimes determined experimentally for the best results. Because of the risk of embolism, air bubbles in fluid and syringe and needle must be purged. Air bubbles can be purged either by advancing the plunger back and forth and/or gently tapping the side of the syringe and slowly expelling the air into sterile absorbent tissue until fluid appears at the tip of the needle. Prior to lateral tail vein injection, the tail should be warmed up with a lamp or by immersing the tail into warm water (40°-45° C) to provide better vessel dilation. Swab the tail with 70% alcohol on gauze. Insert the needle parallel to the tail vein penetrating 2-4 mm into the lumen while keeping the bevel of the needle face upwards. The solution is then injected slowly and no significant resistance should be felt if the solution is properly administered. Since the injectable temporarily replaces the blood in the tail vein, a white streak can be visible under observation. In case of a certain resistance and no blood clearance during administration, it can be possible that the needle is not in the vein but in the surrounding tissue. The needle should be advanced in such a way that it then enters the vein or a new try must be made. When the administration is finished, the injection site must be pressed firmly with a swab to prevent backflow of

the administered volume and/or blood. If the same vein must be used several times, the first administration should be made as distal as possible in relation to the heart and subsequent administrations should be placed progressively more proximally.

Other routes of eXIA™160*XL* administration, e.g. via the external jugular vein, the dorsal metatarsal vein, the sublingual vein, or the femoral vein can be exploited.

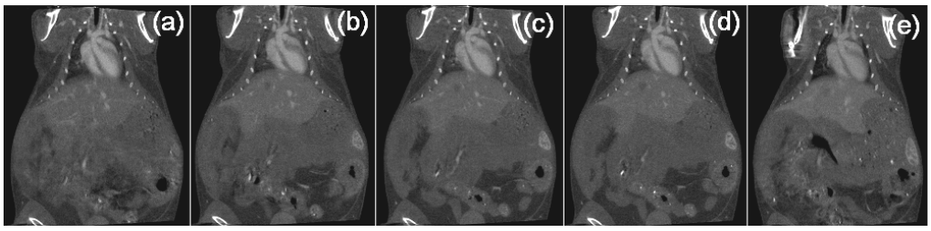
Compatibility of eXIA™160*XL* with the coadministration has not been established, but to reduce the frequency and volume of i.v. injected materials (e.g. coadministration with isotopes for PET or SPECT or fluorophores for optical imaging), compatibility has been shown with 5% Dextrose, Lactated Ringers, Lactated Ringers and 5% Dextrose, 5% Dextrose and 0.2-0.45% Sodium Chloride.

HANDLING AND STORAGE

eXIA™160*XL* is provided in a multi-use glass container at a total volume of 2 mL. Do not freeze and store at 4° to 18° C (40° to 70° F) protected from light. Inadvertently frozen material should not be used. The presence of oxygen and storage at temperatures outside the recommended storage temperature range can be detrimental to the product. Prior to use, invert the vial to make sure the bottom of the glass container becomes visibly clear. Do not use in case of separation of phases. Do not shake the vial. When the vial is open, write the date of the first entry and store at 4°C (40°F). Strict aseptic technique must always be maintained during handling of the agent since it can support the growth of microorganisms if contaminated. Do not use and discard properly if contamination is suspected. eXIA™160*XL* should not be used after the expiration date shown on the vial label. Dilution or mixing with other reagents prior to injection should be avoided unless experimentally proven not to affect the properties of the product.

* Organ enhancement values were determined in normal BALB/c mice following eXIA™160*XL* i.v. administration at 5 mL per kg of body weight. The enhancement values can be affected by the type, duration and degree of anaesthesia, and the health or dietary status of the animal under study.

eXIA™160*XL* Time-Dependent Organ Attenuation



Coronal sections 10 min (a), 20 min (b), 30 min (c), 40 min (d), and 60 min (e) after an intravenous dose* of eXIA™160*XL*.

RELATED PUBLICATIONS

1. Kojonazarov B, Belenkov A, Shinomiya S et al. Evaluating Systolic and Diastolic Cardiac Function in Rodents Using Microscopic Computed Tomography. Circ Cardiovasc Imaging. 2018 Dec;11(12)

2. van Deel ED, Yanto Ridwan Y, et al. In Vivo Quantitative Assessment of Myocardial Structure, Function, Perfusion and Viability Using Cardiac Micro-computed Tomography. J Vis Exp. 2016 Feb 16;(108):53603

3. van Deel ED, Yanto Ridwan Y, et al. Multimodal in vivo Computed Tomography and Optical Imaging for Simultaneous Functional, Metabolic and Molecular Myocardial Analysis. Circulation. 2014;130:A15015

4. Ashton JR, Befera N, Clark D et al. Anatomical and functional imaging of myocardial infarction in mice using micro-CT and eXIA 160 contrast agent. Contrast Media Mol Imaging. 2014 Mar-Apr;9(2):161-8

5. Detombe SA, Dunmore-Buyze J, Drangova M. Evaluation of eXIA 160XL cardiac-related enhancement in C57BL/6 and BALB/c mice using micro-CT. Contrast Media Mol Imaging. 2012 Mar-Apr;7(2):240-6.

6. Prajapati SI, Keller C. Contrast enhanced vessel imaging using microCT. J Vis Exp. 2011 Jan 27;(47). pii: 2377.

CONTACT INFORMATION

For further information on the product, please visit our website at http://www.binitio.com Binitio Biomedical, Inc.
275 Slater Street, Suite 900 Ottawa, ON K1P 5H9, CANADA

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