

Instruction for use

Intibio Alpha-2 MacroGlobulin (A2MG) Kit

Introduction

The A2MG kit is intended for the processing of equine blood for veterinary use only by those who have received adequate training and knowledge on how to properly use orthobiologics in horses. The kit consists of medical grade disposable items. Anticoagulant ACD-A is required for use of this kit and is included.

A2MG Kit Instructions

Step 1: blood collection

- ◆ Fill 1x 50ml syringe (for 15ml kit) or 2x 50ml syringes (for 30ml kit) without luer lock with 5ml each of sterile ACD-A anticoagulant.
- ◆ Prepare the horse's jugular vein for aseptic blood collection.
- ◆ Aspirate 35 ml of blood into one (for 15ml kit) or two (for 30ml kit) 50 ml syringes until they are filled to 40 ml.
- ◆ Rotate the syringes several times so that the blood is well mixed with the anticoagulant.

Step 2: centrifugation to remove blood cells from the plasma

- ◆ Fill all blood tubes with the collected blood.
- ◆ Place the red blood tube holders opposite to each other in the centrifuge.
- ◆ Place the blood tubes in these holders.
- ◆ Centrifuge at 3400 RPM for 10 minutes.
- ◆ After centrifugation, disinfect the septum of the blood tubes with the supplied alcohol wipe.
- ◆ Connect the vacutainer holder to the 50 ml syringe with luer lock.
- ◆ Aspirate all plasma from the blood tubes into this 50 ml syringe.

Step 3: centrifugation to purify for A2MG

- ◆ Carefully divide the plasma into the supplied one (for 15ml kit) or two (for 30ml kit) filter tubes with pierceable yellow cap for ultracentrifugation.
- ◆ Place the tube holders opposite to each other in the centrifuge.
- ◆ Place the filled tube (for 15ml kit) or tubes (for 30ml kit) in these holders.
- ◆ Place the non-sterile centrifuge balance tube (blue cap, for 15ml kit) opposite to the filter tube (yellow cap) or place the filter tubes with yellow cap (for 30ml kit) opposite to each other in the centrifuge. Do not use the non-sterile balance tube for blood processing.
- ◆ Centrifuge at 3400 RPM for 10 minutes.
- ◆ After centrifugation, disinfect the septum of the filter tube(s) with the supplied alcohol wipe.
- ◆ Aspirate the plasma from the filter tube(s) with a long needle into a 20 ml syringe.
- ◆ Connect the red adapter to the syringe for easy distribution to other syringes.
- ◆ Use sterile gloves and aseptically prepare the application site.

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Indication and follow-up:

- ◆ A2MG can be used in the acute or chronic phase.
- ◆ A veterinary clinical follow-up is recommended 2 to 3 weeks joint application.
- ◆ Repeated use is upon discretion of the veterinarian.

Following volumes are recommended:

- ◆ Wound dressing: 4-6ml per application depending on wound size
- ◆ Nebulization: 4ml + 4ml sterile saline
- ◆ Navicular bursa: 2ml
- ◆ Coffin/Pastern/Fetlock: 4ml
- ◆ Elbow/shoulder: 4-6ml
- ◆ Lower hock joints (TMT/TIT): 2ml
- ◆ Talocrural joints: 4ml
- ◆ Stifle joint: 6ml
- ◆ Sacro-iliac joint: 6-10ml
- ◆ Cervical facet joints: 2ml
- ◆ Tendon/ligament intralesional: 1-2ml
- ◆ Tendon/ligament perilesional: 4-6ml

Practical considerations:

- ◆ Do not use the kit in horses with deviating/abnormal blood levels, blood diseases, septicemia or infectious diseases as they may influence the A2MG product and joint/tendon physiology.
 - ◆ A one day wash-out period is recommended after using Alpha-2 agonist sedatives (such as detomidine, romifidine, xylazine) and before preparing A2MG as plasma levels of alpha-2 Macroglobulin may be affected.
 - ◆ If sedation is needed before blood aspiration for A2MG preparation, then the intramuscular route is most preferred.
 - ◆ Regional or intra-articular anesthesia is allowed before blood aspiration.
- A wash-out period according to the product information leaflet is recommended when (N)SAIDs have been systemically administered before blood aspiration for A2MG preparation. When needed for animal welfare reasons, a one-day wash-out is possible.

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Handling Instructions

- ◆ In case no centrifuge is available in the field, the blood should be processed within 4 hours after aspiration to avoid haemolysis and protein degradation. It has to be noted that the blood needs to be maintained in aseptic conditions at all times.
- ◆ After blood aspiration for A2MG preparation, sedation or systemic (N)SAIDs are allowed.
- ◆ After A2MG preparation, use the plasma within 4 hours at room temperature or 12 hours refrigerated. Product must be warmed to body temp (37°C) before use.
- ◆ Unused plasma can be stored frozen in sterile bags using sterile syringes with a sterile cap at -20°C or -80°C for up to 12 months.
- ◆ Make sure the A2MG containing plasma is maintained in aseptic conditions at all times which is crucial to prevent contamination and infection during collection, handling, and storage.
- ◆ After freezing, thaw in the palm of your hand or a warm water bath and heat to 37°C (body temperature) before use. Blood protein clusters or plasma crystals need to dissolve after stirring with a standard vortex and inject within 4 hours after thawing.
- ◆ Make sure the A2MG containing plasma is maintained in aseptic conditions at all times which is crucial to prevent contamination and infection during collection, handling, and storage.
- ◆ Concomitant shockwave, laser, ultrasound or pulsed electromagnetic field therapies are allowed before or 24 hours after local application.

Warnings:

- ◆ The parts cannot be reused and do not use the components if the sterile packaging is damaged.
- ◆ When the content is not transparent or yellow, please do not inject the product and contact the manufacturer.
- ◆ More advanced pathology may result in an increased response after use.
- ◆ Do not mix with other products from animal origin or from different donors.
- ◆ When biological products from different donors are used in the same region a wash-out period of 4 weeks needs to be respected.
- ◆ Do not store syringes in liquid nitrogen.
- ◆ Intended for veterinary use only.

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Recommended protocol for A2MG Kit in equine asthma:

- ◆ Process the horse's blood sample according to the standard procedure specified for the A2MG Kit.
- ◆ Aliquot the final product into 4 mL for dosing.
- ◆ Administer a total of 8 doses at 48- to 72-hour intervals, or until clinical symptoms are adequately controlled. Additional cycles may be initiated if necessary.
- ◆ Standard administration consists of 3-4 mL of A2MG product diluted with an equivalent amount (3-4 mL) of sterile physiological saline (0.9%) or Lactated Ringer's Solution (LRS), resulting in a total volume of 6-8ml per administration. Larger volumes of saline or LRS (to achieve a 1:2 dilution) can be used to reduce the viscosity of the product and facilitate administration (eg 3-4mL of A2MG diluted with 6-8mL of saline or LRS).
- ◆ Delivery of the A2MG can be achieved via nebulization using a Flexineb nebulizer or alternative solution.
- ◆ Horses currently receiving treatment for equine asthma may continue their existing therapy concurrently with A2MG application.
- ◆ Following completion of the initial A2MG regimen, a second cycle could be recommended with a lower volume (2mL A2MG + 6ml dilution) under veterinary supervision.

A2MG nebulization is permitted for use during competition; however, it is critical that the standard preparation and administration protocols are strictly followed to ensure the final product remains free of substances classified as doping agents.

In cases of severe equine asthma, some horses may require additional treatments beyond the initial series to fully recover.

Check the model of nebuliser. Use a medium (grey) cup where possible. Regular maintenance and cleaning of the nebuliser is required.

For further guidance or case-specific recommendations, please contact INTIBIO at info@intibio.com.