vaxkit™

CANCER IMMUNOTHERAPY

Kit for the preparation
of autologous treatment
Veterinarian use only

USER GUIDE

INTRODUCTION

You have just received an **8-dose veterinary autologous personalized immunotherapy preparation kit.**

It takes about two hours to make all eight doses, but you will only be occupied for about one hour.

Three steps are needed to produce eight single doses :

#01 Extraction (#01)

#02 Purification (#02)

#03 Aliquoting (#03)

Followed by labelling and storage.

Sterile gloves must be worn throughout the whole process.

In addition to the sterile single-use equipment contained in this kit, you will also need to use a centrifuge (non provided). Ideally, a 6000 rpm centrifuge for 5 ml tubes is recommended.

However, 4000 rpm with 2 ml tubes is acceptable.

If, however, you need to use smaller tubes, the solution must be broken down into equivalent fractions.

In this case, care must be taken during handling to ensure that the solutions are sterile when transferred.

IMPORTANT: You need to defrost the tumour a few minutes before beginning

Visit https://vaxkit.com/contactus for a video showing how to prepare the kit.

AUTOLOGOUS ANTI-TUMOUR IMMUNOTHERAPY

COMPOSITION for each of the 8 final treatment doses of 0.5 ml :
40 mg hydroxyapatite (+/-10%) ;
<1 mg autologous tumor proteins.
excipient q.s. Carboxymethylcellulose 2% ;

STORAGE :
Keep frozen at -18°C until use.

ADMINISTRATION :
Inject intradermally if not subcutaneously.
Do not inject intravenously.

INDICATION :
Antitumour immunotherapy.

Preferably used after excisional surgery for solid tumours.

May be used during chemotherapy and radiotherapy.

Contains autologous proteins. May only be used on the animal from which it was obtained and whose name is indicated on the doses.

ADVERSE EFFECTS :
Any fever associated with neurological signs suggestive of autoimmune meningoencephalitis should be treated with high-dose corticosteroids and the animal hospitalised.

CONTRAINDICATIONS :
History of autoimmune disease including insulin-dependent diabetes, Canine T-lymphoma.

ADMINISTRATION PROTOCOL :
One dose per week for 4 weeks followed by one dose per month for 4 months.

DRUG INTERACTIONS :
Combination with asparaginase, vincristine, cyclophosphamide, methotrexate,

may lead to autoimmune encephalitis in the treatment of T-cell lymphomas.

In general, it is preferable to avoid immunosuppressive drugs when using immunotherapy.

If steroids are essential, use only low doses if possible.

PACKAGE CONTENT

#01 - bag containing:

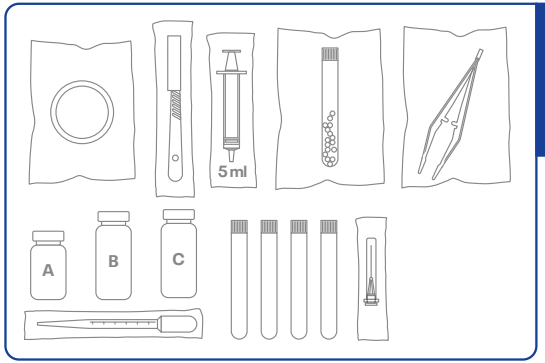
› 1 Petri dish
› 1 scalpel
› 1 5 ml syringe
› Grinding balls
› 1 pliers
› Sterile aqueous solution A / Sodium carbonate 5 ml
› Sterile aqueous solution B / Ammonium sulfate 10 ml
› Sterile aqueous solution C / Phosphate buffer 10 ml
› 1 pipette
› 4 centrifugation tubes
› 1 sterile 23G needle

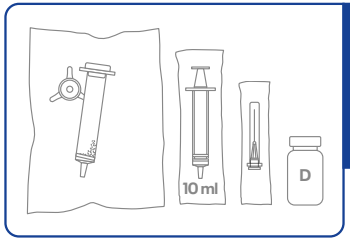
#02 - bag containing:

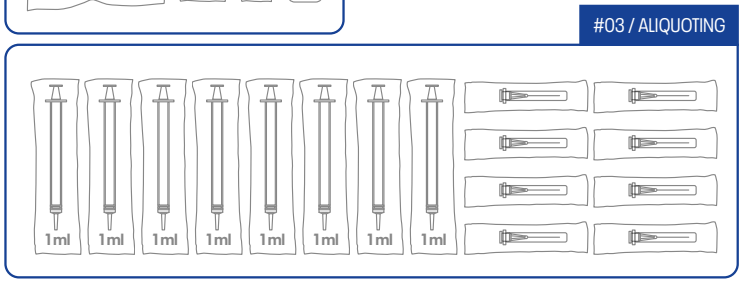
› 1 sterile column: hydroxyapatite content 0.33 g
› 1 sterile 10 ml syringe
› 1 sterile 23G needle
› Sterile aqueous solution D / Carboxy Methyl Cellulose 5 ml

#03 - plastic box containing:

› 8 sterile 23G needles
› 8 sterile 1 ml syringes
› 8 small labels
› 1 large format label







SUMMARY

#01

EXTRACTION

- Prepare the solution for homogenization
- Homogenize the tumour
- Centrifuge & keep the supernatant
- Cryoprecipitation
- Centrifuge a second time & keep the pellets
- Produce the extraction solution

#02

PURIFICATION

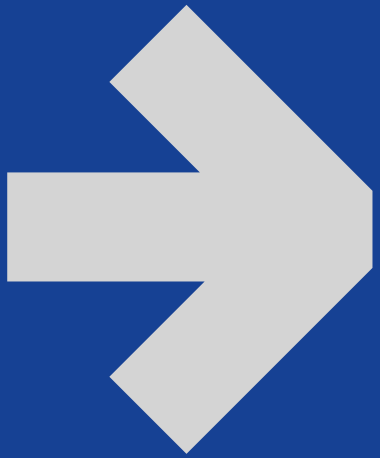
- Transfer the extraction solution into the chromatography column
- Rince the column with C solution
- Add D solution to the column

#03

ALIQUOTING

- Divide the treatment into 8 single doses
- Individual labelling of the 8 single doses and their storage box

PREPARING BEFORE INJECTION



#01 EXTRACTION

Start by opening and checking the contents of the bag marked #01 EXTRACTION, which gathers all the sterile, single-use components required for this step.

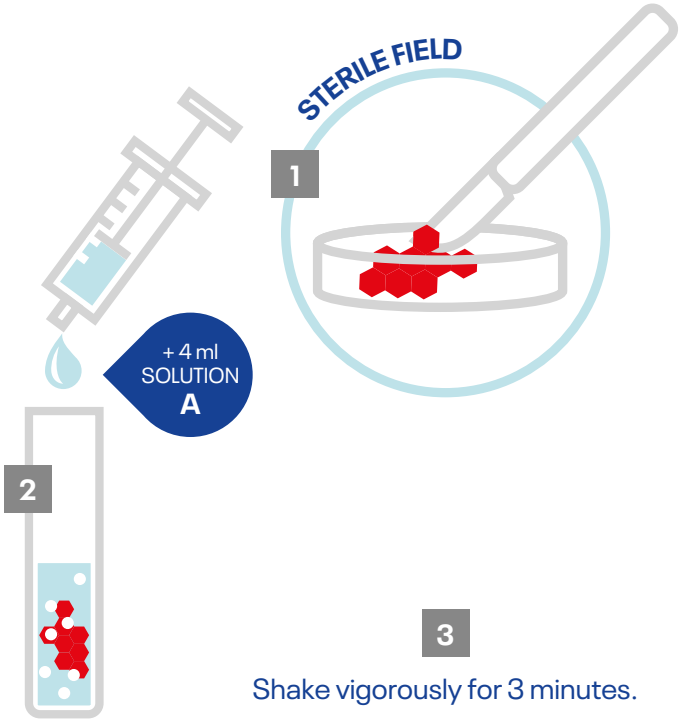
#01 EXTRACTION

#01.1 PREPARATION OF THE TUMOUR CELL LYSIS SOLUTION

A solution makes tumour cells burst, releasing cytoplasm and membrane proteins.

First place a sterile drape on your work surface:

1. In sterile conditions, extract about 0.5 cm^3 of the tumour and cut it up into small pieces. Place the extract in the sterile tube containing the ceramic balls.
2. Use the 5 ml syringe to add 4 ml of solution A.
3. Shake manually for 3 minutes.



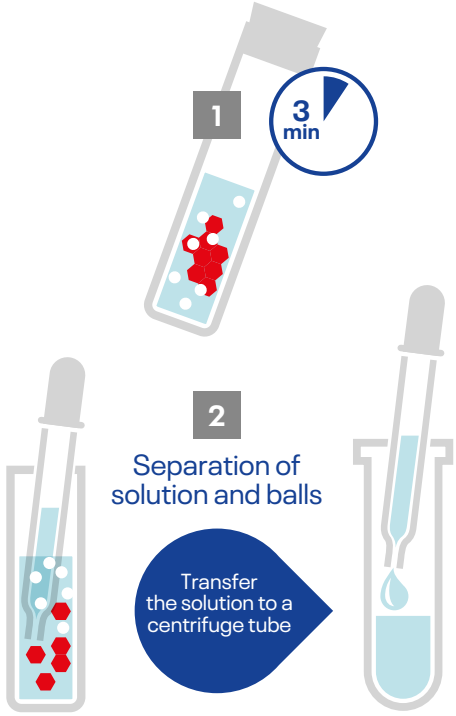
#01 EXTRACTION

#01.2 GRINDING THE TUMOUR

Recovery of the grinding solution in preparation for centrifugation.

1. **Observe** the contents. If necessary, homogenize again until liquid (although tumour residues may remain).
2. **Transfer** all the resulting solution into the centrifuge tube using the sterile pipette provided. Avoid taking any residual tumour fragments.

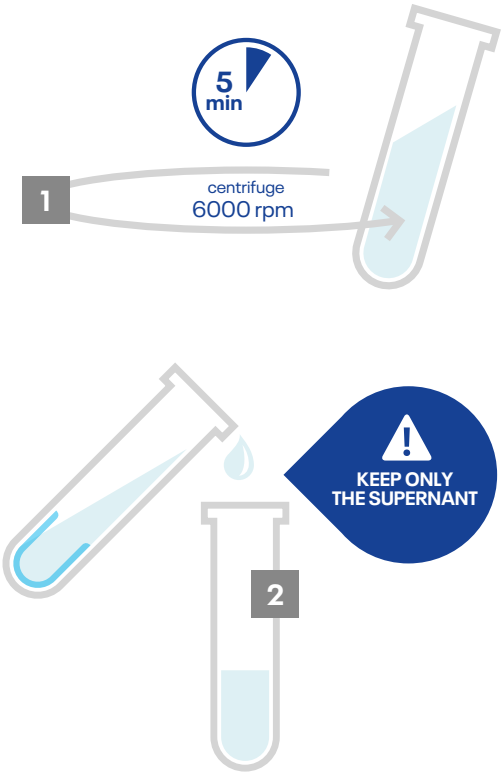
To avoid clogging up the Pasteur pipette, ensure the tip is under the ceramic balls before drawing the solution into it.



#01.3 FIRST CENTRIFUGATION & KEEP THE SUPERNATANT

Centrifugation for removal of cell debris which will be in the pellet.

1. Centrifuge at **6,000 rpm for 5 minutes** to obtain a visible pellet and a liquid phase free of solid supernatant.
2. **Keep the liquid supernatant** and transfer it into a new sterile centrifuge tube.

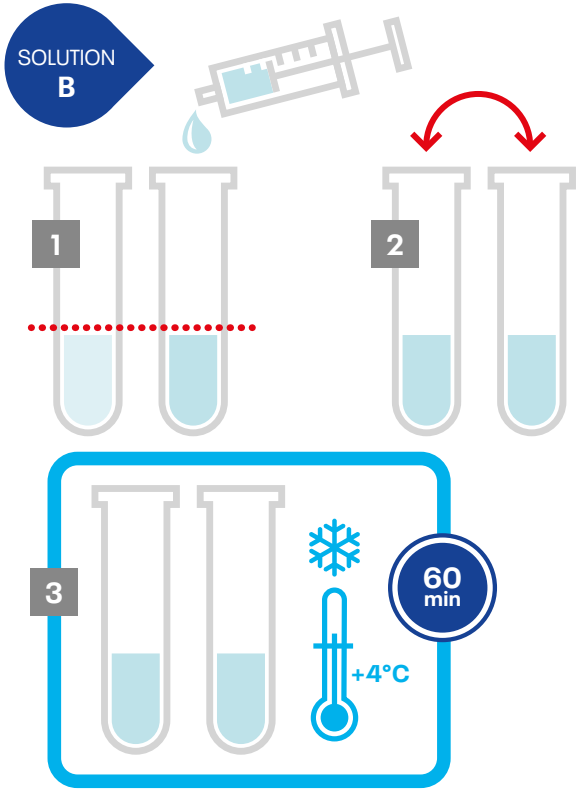


#01 EXTRACTION

#01.4 CRYOPRECIPITATION

Protein precipitation at low temperature using solution B.

1. Use the 5 ml syringe to place **an equal quantity of solution B as the supernatant from the previous step** into a new sterile centrifuge tube.
2. Mix the content of the two tubes together by transferring them from one tube to the other at least six times until a homogeneous solution is obtained in both tubes.
3. Refrigerate the two tubes (placed vertically) at **+4°C for 60 minutes.**

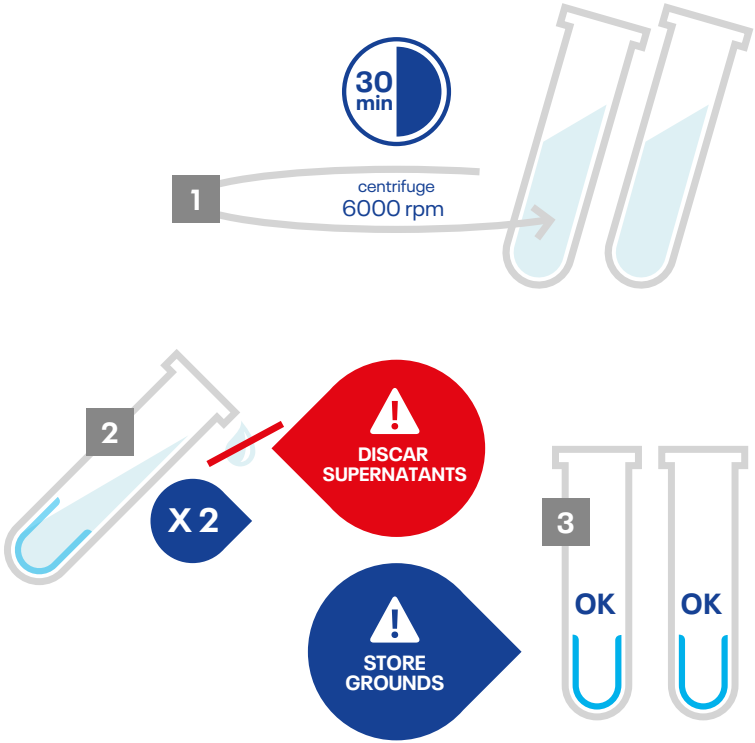


#01.5 CENTRIFUGE A SECOND TIME & KEEP THE PELLETS

Centrifugation to concentrate the proteins of interest from cryoprecipitation.

When removed from the refrigerator, a precipitate appears in the tubes.

1. Centrifuge the two identical tubes at **6,000 rpm for 30 minutes.**
2. Discard the supernatant from both tubes.
3. **Retain the pellets.**

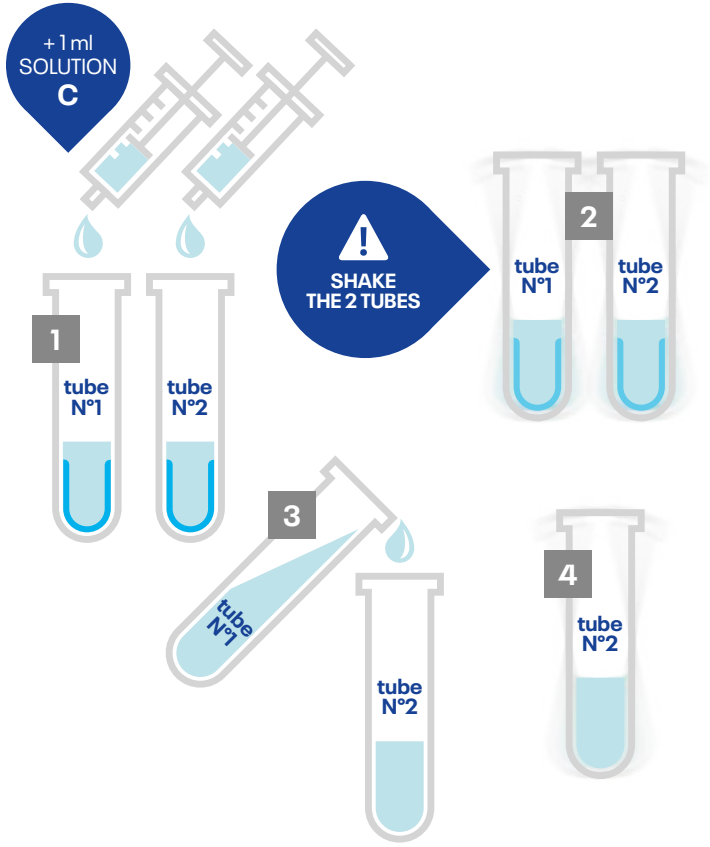


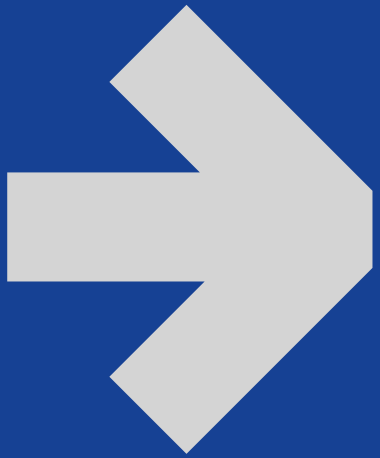
#01.6 PRODUCE EXTRACTION SOLUTION

Protein pellet dissolved in buffer solution C.

1. Use the 5 ml syringe to **add 1 ml of solution C to the pellet in each of the two tubes.**
2. Shake until the pellets are fully dissolved.
3. Transfer the contents of tube 1 into tube 2.
4. Shake.

PLEASE NOTE
you will need the end of vial C to carry out the PURIFICATION step





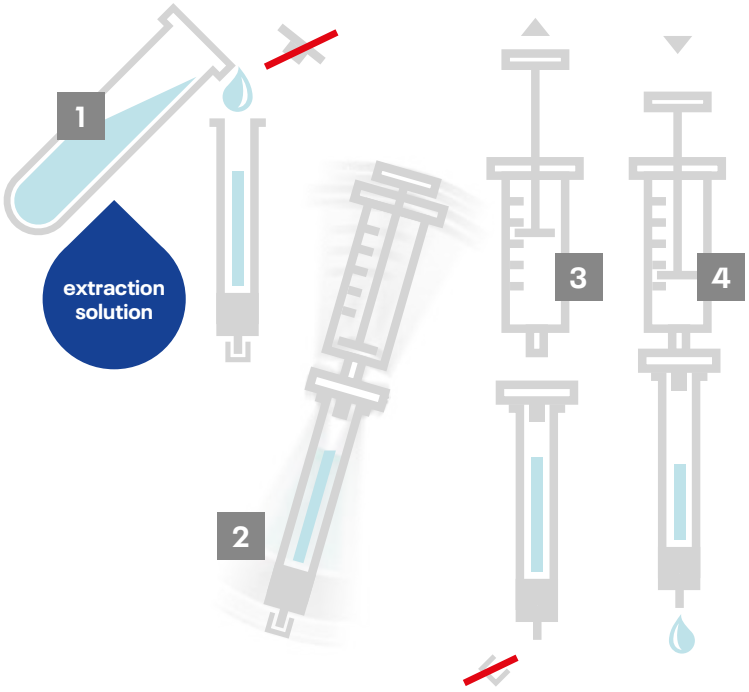
#02
PURIFICATION

The bag identified as #02 PURIFICATION contains the sterile, single-use components required for this step.
IMPORTANT: You will also need the remaining content of solution C from the previous bag identified as #01 EXTRACTION.

#02.1 TRANSFER THE EXTRACTION SOLUTION INTO THE CHROMATOGRAPHY COLUMN

The hydroxyapatite column chromatographically selects and concentrates the proteins of interest contained in the extraction solution.

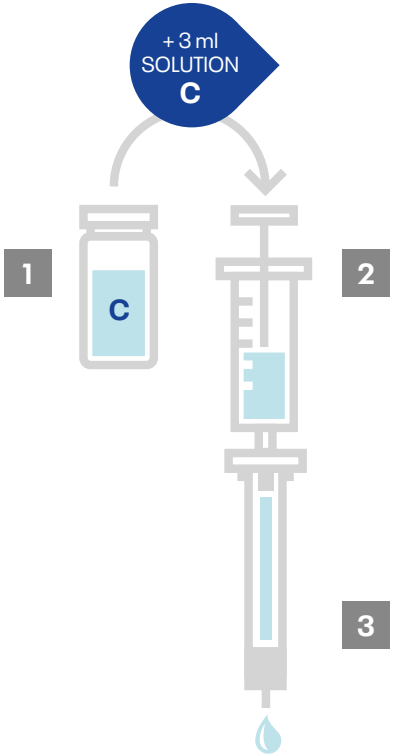
1. **Put the plug** at the bottom of the column and uncap the top of the column, shake the extraction tube if necessary and pour its contents into the column.
2. **Cap the top** of the column with the adaptor and 10 ml syringe (without the needle and with the plunger in position). Shake vigorously. Allow the powder to settle (3 min).
3. **Remove** the syringe, fill it with air then put it back on the adaptor. Uncap the bottom of the column.
4. **Slowly depress** the plunger so that all the liquid flows down through the powder. Let the extra liquid drain out.



#02.2 RINSE THE COLUMN WITH SOLUTION C

The remaining Buffer C is used to eliminate proteins that have not bound to the hydroxyapatite column.

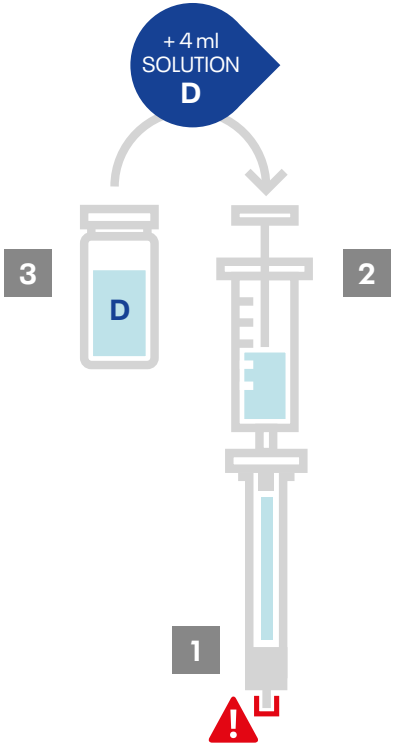
1. Use the 10 ml syringe fitted with a needle **to extract 8 ml of solution C.** Remove the needle then add this solution into the upright column.
2. Slowly depress the plunger so that all the liquid flows down through the upright column. Let any extra liquid drain out (2 min).
3. Remove the syringe, draw in 5 to 10 ml of air then slowly inject it into the column and let the extra liquid drain out for a further 2 min. Remove the syringe. Repeat as required.

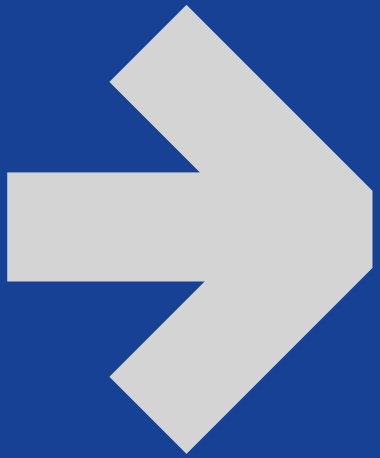


#02.3 ADD D SOLUTION TO THE COLUMN. RESUSPENSION OF PROTEIN-LOADED HYDROXYAPATITE GRAINS

Solution D is designed to resuspend the hydroxyapatite. It allows the treatment to be injected without pain.

1. Cap the bottom of the column.
2. Add a needle to the 10 ml syringe, **draw in 4 ml of solution D**.
3. Then depress the plunger to add the solution to the column.





#03 ALIQUOTING

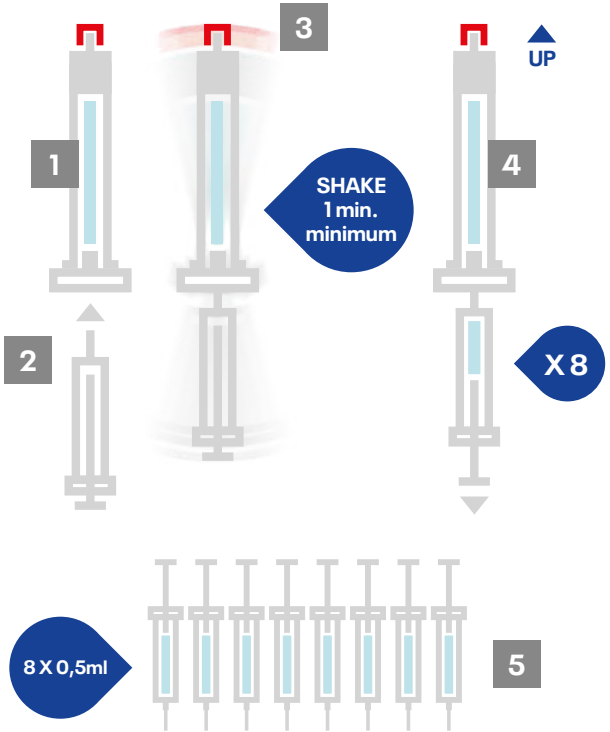
The box identified #03 ALIQUOTAGE contains 8 sterile syringes intended to receive the 8 vaccine doses aliquoted from the contents of the column (4 ml of hydroxyapatite suspended in solution D). They are labelled and stored at -18°C.

#03 ALIQUOTING

#03.1 DIVIDE THE TREATMENT INTO 8 SINGLE DOSES

The suspension of hydroxyapatite grains must be taken from the chromatographic column and divided into 8 homogeneous syringes of the same volume (0.5 ml).

1. **Block** the column with the 10 ml syringe without needle To facilitate resuspension, suck and push repeatedly to 'loosen' the block of powder formed while keeping the filter in place. The column should remain upright, so as not to aspirate its contents. Tap the body of the column lightly on the edge of a table to tip the powder block. When the block of powder has lifted off, shake vigorously by hand to obtain a homogeneous suspension
2. **Take** a 1 ml syringe from the single dose storage box. Attach this syringe to the column adaptor to block it
3. **Shake** vigorously the column until the powder is completely resuspended (between 30 sec and 60 sec).
4. **Invert** the column and aspirate 0.5 ml of the preparation, cap the syringe with a needle and place it in the storage box for the single doses.
5. **Proceed** in the same way 7 more times, shaking strongly before each aspiration (10 sec. minimum).

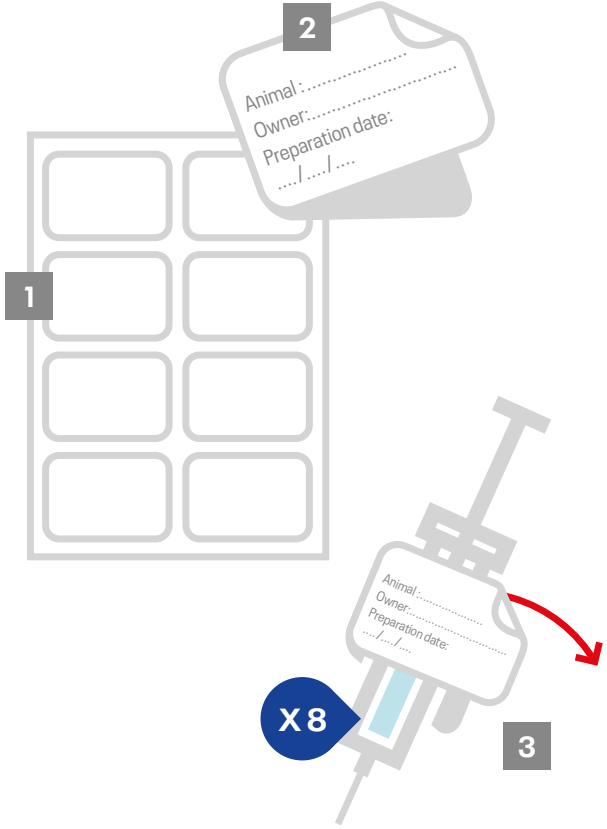


#03.2 INDIVIDUAL LABELLING OF THE 8 SINGLE DOSES AND THEIR STORAGE BOX

As soon as they have been prepared, the 8 monodoses must be individually identified.

1. **Use** the individual labels provided
2. **Write** the PET's NAME, the OWNER's NAME and the DATE of preparation on all 8 labels.
3. **Wrap** the label around the top of each syringe.

Repeat this operation for all 8 syringes.



#03 ALIQUOTING

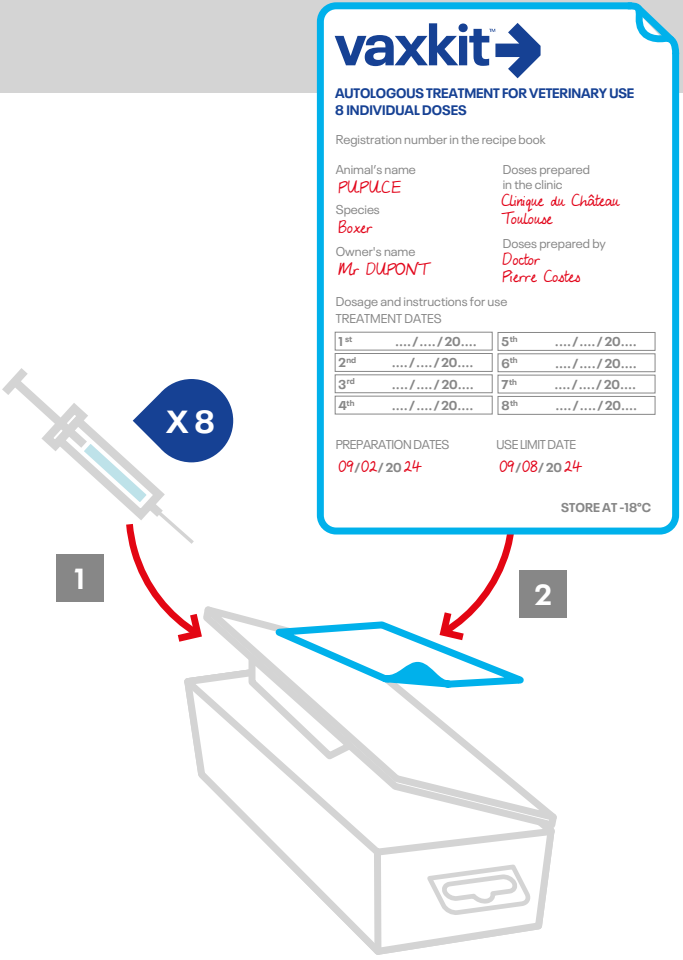
1 **Place** the 8 individual doses in the storage box

2 **Write** the following information on the large label:

› **For the pet :**
- PET's NAME
- OWNER's NAME

› **For the treatment**
- DATE of preparation
- PLACE of preparation
- NAME of the person who prepared the treatment

Place the storage box in a freezer at -18°C



#03.3 PREPARING BEFORE INJECTION

1. Prior to injection, **allow to defrost** for 5 to 10 minutes.
2. **Draw in** 0.5 ml of air and shake well for at least 20 seconds to obtain a homogeneous suspension
3. **Evacuate** the air then inject subcutaneously in accordance with standard procedures.

Ensure the syringe is perfectly vertical.

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CANCER IMMUNOTHERAPY

Hastim

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